Diploma Thesis

Isavuconazole Treatment in a Mixed Patient Cohort with Invasive Fungal Infections: Outcome, Tolerability and Clinical Implications of Isavuconazole Plasma Concentrations

submitted by

Maximilian Waller

for the academic degree of

Doktor der gesamten Heilkunde

(Dr. med. univ.)

at the

Medical University of Graz

conducted at the

Division of Infectious Diseases and Tropical Medicine/

Department of Internal Medicine Graz

under the guidance of

Univ.-Prof. Dr. med. univ. Robert Krause

Dr. med. univ. Christoph Zurl

Graz, am 3. Dezember 2020

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 3. Dezember 2020 *Maximilian Waller eh.*

Danksagungen

Ein Dankeschön gilt meinen Betreuern für die Diplomarbeit, Univ.-Prof. Dr. med. univ. Robert Krause und Dr. Christoph Zurl, welche mir eine wertvolle Stütze bei der Erstellung dieser Diplomarbeit waren und mir jederzeit mit schneller, kompetenter und unkomplizierter Hilfe zur Seite standen.

Meiner Familie möchte ich für die Unterstützung danken, auf welche ich nicht nur während des Studiums, sondern in allen Lebenslagen bisher vertrauen durfte.

Einen wesentlichen Beitrag zu meiner erfolgreichen Studienzeit haben meine Freunde geleistet. Auch ihnen gilt ein besonderer Dank!

Table of Contents

List of Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
AmB	Amphotericin B
AML	Acute myeloid Leukemia
ARDS	Acute Respiratory Distress Syndrome
BAL	Bronchoalveolar Lavage
BDG	1,3-Beta-D Glucan
BMI	Body-Mass-Index
C_{max}	Maximum Concentration
CRRT	Continuous Renal Replacement Therapy
CSF	Cerebrospinal Fluid
CYP	Cytochrome P450
DNA	Deoxyribonucleic Acid
ECMO	Extracorporeal Membrane Oxygenation
EMA	European Medicines Agency
EORTC	European Organization for Research and Treatment of Cancer
FDA	Food and Drug Administration
HSCT	Hematopoietic Stem Cell Transplantation
IA	Invasive Aspergillosis
IDSA	Infectious Diseases Society of America
ICU	Intensive Care Unit
IFD	Invasive Fungal Disease
IQR	Interquartile Range
ISA	Isavuconazole
ITT	Intention-to-treat
lipAmpB	Liposomal Amphotericin B
MMF	Mycophenolic Acid
MSG	Mycoses Study Group
PCR	Polymerase Chain Reaction
PPK	Population Pharmacokinetic
RRT	Renal Replacement Therapy
Spp.	Species Pluralis
STAT 3	Signal Transducer and Activator of Transcription 3

TDM	Therpeutic Drug Monitoring/Therapeutisches Drug Monitoring
TEAE	Treatment-Emergent Adverse Event
UGT	Uridine Diphosphate Glucuronosyltransferase

List of Figures

List of Tables

Zusammenfassung

Einleitung

Isavuconazol (ISA) ist ein Breitspektrum-Triazol-Antimykotikum mit einem günstigen pharmakokinetischen Profil, das für den Einsatz bei Patientinnen und Patienten mit invasiver Aspergillose oder Mukormykose zugelassen ist. Bisherige Studien weisen darauf hin, dass ein Therapeutisches Drug Monitoring (TDM) möglicherweise nicht notwendig ist, jedoch gibt es kaum Daten in der alltäglichen Anwendung von ISA. Ziel dieser Studie war es, ISA-Plasmaspiegel unter Alltagsbedingungen bei einer heterogenen Patientenkohorte zu bestimmen und die Ergebnisse in Verbindung mit der Wirksamkeit und Sicherheit zu bewerten.

Methoden

Eine Studiengruppe der Medizinischen Universität Graz untersuchte die ISA Serumspiegel einer gemischten Patientenkohorte, die mit ISA behandelt wurden. Die Studie umfasste 33 ISA-Behandlungen an 32 erwachsenen Patientinnen und Patienten mit hämatologischen und anderen Grunderkrankungen und bewertete das klinische Ansprechen, die Nebenwirkungen und die ISA-Plasmatalspiegel.

Ergebnisse

Ein vollständiges oder teilweises Therapieansprechen wurde bei 87% der Patientinnen und Patienten festgestellt und ISA wurde insgesamt gut vertragen. Die mediane ISA-Plasmakonzentration betrug 3.05μg/ml (IQR 1.93-4.35) bei Patientinnen und Patienten ohne Nierenersatztherapie (RRT) oder extrakorporaler Membranoxygenierung (ECMO) und war bei Patientinnen und Patienten mit RRT einschließlich der Fälle mit zusätzlicher ECMO- oder Cytosorb® Adsorbertherapie signifikant niedriger (0.88μg/ml, IQR 0.71-1.21).

Diskussion

Zusätzlich zu früheren Empfehlungen schlagen wir vor ISA Talspiegel in speziellen Patientengruppen einschließlich derer mit RRT und anderen extrakorporalen Behandlungsverfahren, sowie bei Patientinnen und Patienten mit Adipositas mittels TDM zu überwachen.

Abstract

Background

Isavuconazole (ISA) is a broadspectrum triazole antifungal agent with a favorable pharmacokinetic profile indicated for use in patients with invasive aspergillosis (IA) or mucormycosis. Previous studies have indicated that therapeutic drug monitoring (TDM) might not be necessary in daily clinical practice but supporting data in real-life use is scarce. The objective of this study was to evaluate ISA levels in a real world setting in a mixed patient cohort and to correlate findings with efficacy and safety outcomes.

Methods

A study group of the Medical University of Graz evaluated ISA serum levels in a mixed patient cohort undergoing ISA treatment. The investigation included 33 ISA treatment courses in 32 adult patients with hematological and other underlying diseases and assessed the clinical response, side effects, and ISA trough plasma concentrations.

Results

Complete response or partial treatment response was found in 87% of the patients, and ISA was generally well tolerated. The median ISA plasma concentration was 3.05μg/ml (IQR 1.93-4.35) in patients without renal replacement therapy (RRT) or extracorporeal membrane oxygenation (ECMO) and significantly lower in patients with RRT, including cases with additional ECMO or Cytosorb® adsorber therapy (0.88μg/ml, IQR 0.71-1.21).

Conclusion

In addition to previous recommendations, we propose to monitor ISA plasma concentrations in special patient groups, including RRT, other extracorporeal treatments, and obesity.

1 Introduction

Isavuconazole (ISA) is a broad spectrum second-generation triazole indicated for use in the treatment of invasive aspergillosis (IA) and mucormycosis (1,2). In the spectrum of invasive fungal disease (IFD), triazole antifungal agents represent an important category of drugs for prophylaxis and treatment. The introduction of ISA on the market is an advance in the complex treatment of IFD.

The SECURE study showed ISA to be non-inferior in efficacy compared to voriconazole for the primary treatment of invasive mold disease due to *Aspergillus spp*. or other filamentous fungi, with a significantly lower rate of drug-related adverse events (1). Based on this study, the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) licensed ISA to treat IA in 2015 (3,4).

The VITAL study assessed ISA's efficacy and safety for the treatment of mucormycosis and compared its efficacy to Amphotericin B (AmB) in a matched case-control analysis (2). On the outcomes of the VITAL study the EMA licensed ISA for the treatment of invasive mucormycosis in patients for whom AmB is inappropriate (3). The FDA licensed its use for the treatment of invasive mucormycosis in general (4).

The main purpose of the following work was to look at ISA trough plasma concentrations observed in a mixed group of patients to acquire further data in a real-life setting regarding therapeutic drug monitoring (TDM), efficacy, and safety in patients receiving treatment with ISA. This thesis was generated in the context of an observational national multicenter cohort study that included 33 treatment courses of ISA and reports the study thoroughly.

The first part of the thesis describes IFD in general, gives an overview of antifungal therapy, and reviews ISA with its pharmacological details, current guideline recommendations, and TDM in antifungal management. In the second part the study is presented. Previous studies have indicated that TDM for ISA might not be necessary due to its pharmacodynamics, but supporting data in real-life use reflecting a more varied patient population is scarce (5).

1.1 Invasive fungal disease

Fungi are eukaryotic organisms and make up a kingdom of themselves in the taxonomic classification of biology next to Animalia, Plantae, Protista, and Monera (6). An estimation

of nearly four million different fungal species has recently been proposed after modern technological advances such as genetic sequencing and computational sciences found its way into exploring the mycobiome (7). Around 300 of these species were described to cause disease in human beings, but only a small group of around 25 of these species account to a majority of infections in humans (8). It is further interesting to note that the number of fungal infections has been increasing worldwide (9). For example, mortality attributed to IFD rose significantly in the United States of America (USA) between 1980 and 1997 (10). The rising incidence of IFD can be explained with a rising number of patients at risk, namely immunosuppressed patients (11).

The medical nomenclature in german-speaking countries divides pathogenic fungi into the three major groups of dermatophytes (D), yeasts (H), and molds (S), comprising a system called DHS (12). This system was first described by Hans Rieth and did not correctly reflect the fungal system's biological nomenclature, but it provides a simple, clinically oriented classification (13). Other groups of pathogenic fungi that are not represented by the DHS system are dimorphic fungi and the dematiaceous fungi.

While dermatophytes primarily cause superficial fungal infections of skin and skin adnexa representing the most common fungal infection type, molds and yeasts can cause a large variety of fungal infections, occasionally inducing IFD in a population at risk. IFD is associated with substantial morbidity and mortality. Despite recent advances in diagnostic approach and antifungal drugs, diagnosis and treatment of IFD remains difficult (14,15).

Common yeasts that cause IFD are *Candida spp.*, *Cryptococcus spp.* and less frequently encountered ones such as *Saccharomyces spp.*, *Malassezia spp.,* and *Geotrichu candidum*. Molds that frequently cause IFD are *Aspergillus spp*., *Fusarium spp*., *Scedosporium prolificans*, *Mucor*, *Rhizopus*, *Rhizomucor,* and *Absidia* (Table 1).

Table 1. Common invasive fungal diseases, causative fungal species and treatment options (8).

Disease	Fungal species	Treatment options
Invasive Aspergillosis	*Aspergillus fumigatus* *Aspergillus flavus* *Aspergillus terreus* *Aspergillus calidoutus*	Azoles, Polyenes, Echinocandines

Mucormycosis	*Rhizopus spp*. *Mucor spp*. *Cunninghamella bertholletiae*	Polyenes, Azoles
Dimorphic mycoses	*Blastomyces dermatitidis* *Cocciodoides immitis* *Coccidioides posadasii*	Azoles, Polyenes
Disseminated Cryptococcosis	*Cryptococcus neoformans* *Cryptococcus gattii*	Amphothericin B in combination with 5-flucytosin
Invasive candidiasis	*Candida albicans* *Candida tropicalis* *Candida glabrata* *Candida parapsilosis*	Echinocandins, Azoles, Polyenes

Definitions of IFD have been introduced by the *European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC)* and the *Mycology Study group (MSG)* of the *National Institute of Allergy and Infectious Diseases (NIAID)* in an effort facilitate clinical research. Host factors, clinical manifestations, and mycological criteria are the basis of these definitions. A hierarchic structure reflects the level of certainty for diagnosis expressed by the terms "proven", probable" and "possible" (16).

Proven invasive fungal disease requires the detection or culture of fungi from sterile material using biopsy, needle aspiration, blood culture or any other sterile procedure. **Probable invasive fungal disease** is defined by underlying host factors, clinical signs and symptoms, and mycological criteria, as stated in the latest revision of the definition by the *EORTC/MSG* (17). In the absence of mycological criteria, but with the appropriate host factors as well as the clinical signs and symptoms, the definition **possible invasive fungal disease** is applicable. The criteria for invasive mold infections are summarized in Table 1.

Table 2. Diagnostic criteria for invasive mold disease (17).

Proven Invasive Aspergillosis (one of the following)
Microscopic Analysis: Sterile Material

Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast like forms
Culture: Sterile Material
Recovery of a hyaline or pigmented mold by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL fluid, a paranasal or mastoid sinus cavity specimen, and urine
Blood
Blood culture that yields a mold in the context of a compatible infectious disease process
Serology
Not applicable
Tissue Nucleic Acid Diagnosis
Amplification of fungal DNA by polymerase chain reaction (PCR) combined with DNA sequencing when molds are seen in formalin-fixed paraffin embedded tissue
Probable invasive aspergillosis (requires the presence of at least 1 host factor, a clinical feature and mycologic evidence and is proposed for immunocompromised patients only)
Host factors - Recent history of neutropenia (<0.5x109 neutrophils/L [<500 neutrophils/mm3] for >10 days) temporally related to the onset of invasive fungal disease - Hematologic malignancy - Receipt of an allogenic stem cell transplant - Receipt of a solid organ transplant - Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a therapeutic dose of ≥ 0.3mg/kg corticosteroids for ≥ 3 weeks in the past 60 days - Treatment with other recognized T-cell immunosuppressants, such as calcineurin inhibitors, tumor necrosis factor-α-blockers, lymphocyte-specific monoclonal antibodies, immunosuppressive nucleoside analogues during the past 90 days

- Treatment with recognized B-cell immunosuppressants, such as Bruton's tyrosine kinase inhibitors, eg., Ibrutinib
- Inherited severe immunodeficiency (such as chronic granulomatous disease, STAT 3 deficiency, or severe combined immunodeficiency)
- Acute graft-versus-host disease grade III or IV involving the gut, lungs, or liver that is refractory to first-line treatment with steroids

Clinical features

Pulmonary aspergillosis (Presence of 1 of the following 4 patterns on CT)

- Dense, well-circumscribed lesion(s) with or without a halo sign
- Air crescent sign
- Cavity
- Wedge-shaped and segmental or lobar consolidation

Other pulmonary mold diseases

- As for pulmonary aspergillosis but also including a reverse halo sign

Tracheobronchitis

- Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis

Sino-nasal diseases

- Acute localized pain (including pain radiating to the eye)
- Nasal ulcer with black eschar
- Extension from paranasal sinus across bony barriers: including into the orbit

Central nervous system infection

- Focal lesions on imaging
- Meningeal enhancement on magnetic resonance imaging or CT

Mycological evidence

- Any mold, for example, Aspergillus, Fusarium, Scedosporium species or Mucorales recovered by culture from sputum, BAL, bronchial brush, or aspirate
- Microscopical detection of fungal elements in sputum, BAL, bronchial brush, or aspirate indicating a mold

Tracheobronchitis

  - Aspergillus recovered by culture of BAL or bronchial brush
  - Microscopical detection of fungal elements in sputum, BAL, bronchial brush, or aspirate indicating a mold

Sino-nasal diseases

○ Mold recovered by culture of sinus aspirate samples ○ Microscopic detection of fungal elements in sinus aspirate samples indicating a mold Aspergillosis only Galactomannan antigen (antigen detected in plasma, serum, BAL, or CSF; any 1 of the following) ○ Single serum or plasma: ≥ 1.0 ○ BAL fluid: ≥ 1.0 ○ Single serum or plasma: ≥ 0.7 and BAL fluid ≥ 0.8 ○ CSF: ≥ 1.0 Aspergillus PCR (any 1 of the following) ○ Plasma, serum, or whole blood: 2 or more consecutive PCR tests positive ○ BAL fluid: 2 or more duplicate tests positive ○ At least 1 PCR test positive in plasma, serum, or whole blood and 1 PCR test positive in BAL fluid Aspergillus species recovered by culture from sputum, BAL bronchial brush, or aspirate
Possible invasive fungal disease
Cases that meet the criteria for a host factor and a clinical feature, but for which mycological evidence has not been found

Only four different genera of fungi account for more than 90% of fungal-related fatalities: *Cryptococcus spp*., *Candida spp*., *Aspergillus spp*., and *Pneumocystis* (18). In Europe, *Candida spp*. and *Aspergillus spp*. are the largest cause of life-threatening IFD (19).

Some of the potential pathogenic fungal species are commensals to humans, and others are widely found in our environment. Still, even though everyday contact with fungal pathogens is extensive, it rarely leads to invasive disease. Resiliency to fungal infections is based on an intact immune system. Thus, most of the healthy population does not develop invasive fungal infection due to their daily exposure to fungal pathogens (18).

1.1.1 Invasive Aspergillosis

IA is a disease caused by *Aspergillus spp*., a pathogenic fungal species categorized as molds found ubiquitous worldwide. *Aspergillus spp*. occur in soils independently of an animal host

and are omnipresent in indoor air. *Aspergillus spp*. are opportunistic pathogens and typically cause invasive disease in immunocompromised hosts. In these hosts, IA is one of the main causes of deaths related to infection, which makes successful prophylaxis an therapy essential (20,21).

Identified risk factors that favor pathogenesis of IA include prolonged neutropenia (<100 neutrophils/μL), hematopoietic stem-cell transplantation, or solid-organ transplantation, patients with advanced acquired immunodeficiency syndrome (AIDS), hereditary immunodeficiency syndromes, chronic granulomatous disease, and patients receiving immunosuppressive agents (21,22). Also, critically ill patients treated in intensive care units (ICU) are at risk of IA (23). Influenza virus infection was found to be an independent risk factor for IA in ICU patients (24).

The main route of an infestation is by inhaling the conidia of *Aspergillus spp*., thus resulting primarily in infections of the lung with potential subsequent fungemia. Of around 200 described species of *Aspergillus*, *Aspergillus fumigatus* most commonly causes infections. Still, other species can also cause invasive fungal disease such as *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus nidulans,* and *Aspergillus versicolor* (12).

The spectrum of disease caused by *Aspergillus spp*. is large (Figure 1) (25). The clinical manifestation of an infection with *Aspergillus spp*. depends on the host's immune response. Clinical signs and symptoms are often quite nonspecific in patients with IA, who might report fever, cough, and dyspnea. After vascular invasion, IA might cause pleuritic pain due to pulmonary infarction or hemoptysis. Fungaemia can lead to disseminated IA involving virtually all organs, including infection of the central nervous system (21,22).

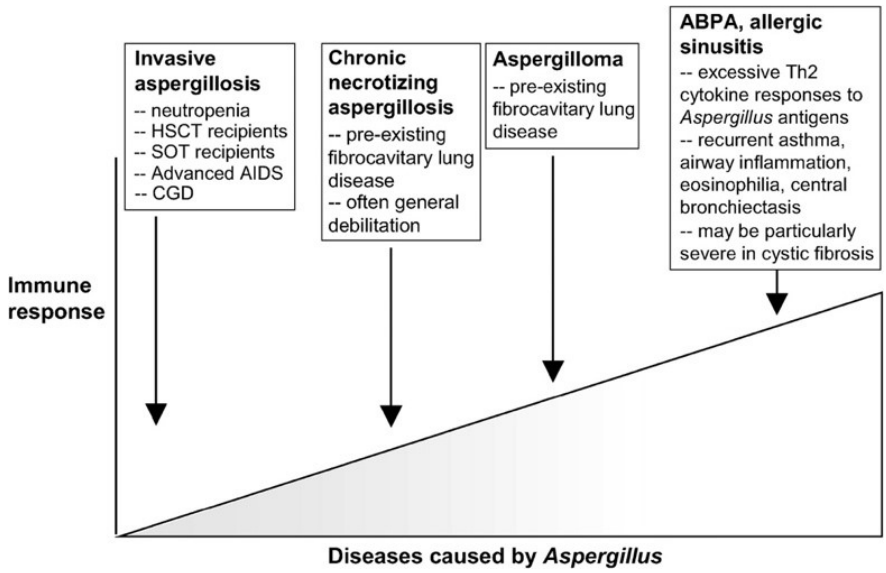

Figure 1. Spectrum of disease resulting after infection with *Aspergillus spp.* Dependent on the status of the host's immune system, different entities of diseases can emerge. Allergic bronchopulmonary aspergillosis (ABPA) and allergic sinusitis result from an atopic disposition in patients with mild or no impairment of the immune system. Aspergilloma is caused by *Aspergillus spp*. chronically colonizing preexisting lung cavities. Aspergilloma and chronic necrotizing aspergillosis can be observed in modestly immunosuppressed patients. Invasive Aspergillosis occurs in severely immunocompromised patients and represents the most severe form of Aspergillus disease.

Fast diagnosis and early initiation of adequate therapy in IA are important to improve outcomes (26). Diagnosis is based on clinical, radiological, and mycological criteria. Definitions of IFD were put forward in 2002 by the EORTC/MSG (16) to establish a comparable basis for clinical research, and revisions were made in 2008 (27). In 2020 another updated revision of the definitions was published (for summarized definitions, see Table 2) to overcome previous shortcomings and establish a version applicable in clinical, diagnostic, and epidemiologic research on a broader range of patients at risk (17). AspICU algorithm for the diagnosis of IA in critically ill patients was published in 2012 and was primarily designed for IA's clinical diagnosis. EORTC/MSG criteria were mainly designed for a uniform entry of patients into studies (22,28). Besides, several expert panels have outlined the diagnostic and therapeutic management of IA in guideline statements (20,29,30).

Histopathologic and cytologic examination of tissue with simultaneous culture is the current gold standard for establishing the diagnosis of IA (29). Also, amplification of fungal DNA by polymerase chain reaction (PCR) combined with DNA sequencing when molds are seen in formalin-fixed paraffin-embedded tissue was recognized as criteria for proven invasive

fungal disease (17). The feasibility of biopsy or needle aspiration in order to retain an adequate amount of specimen for diagnostics in patients with suspected IA is limited.

Biomarkers such as Galactomannan (GM) and 1,3-beta-D-glucan (BDG) are important diagnostic tools in detecting *Aspergillus spp*.. GM is a cell wall component of Aspergillus *spp*. which, when detected in serum or bronchoalveolar lavage fluid (BALF), supports the diagnosis of IA in certain patient subpopulations such as hematologic malignancy or HSCT (29). The available GM assay only is validated for the use in serum or BALF specimen but has prooven its performance in CSF and other specimens (31). Thresholds have been established for adults and children (17). The use of BDG as a marker for IA is recommended in a high-risk population of patients (hematological malignancy, allogenic HSCT), but it is not specific for *Aspergillus* (32). Taken together, biomarkers may not be a stand alone, but a valuable piece of information in the diagnostic process of IA.

A computed tomographic (CT) scan of the chest is recommended for all patients suspected of having IA with high-resolution CT being the preferred method (29). Classical features of IA in the radiological study include dense, well-circumscribed lesion(s) with or without a halo sign (an area of ground glass opacity surrounded by a ring of consolidation), air crescent sign, cavity, or wedge-shaped and segmental or lobar consolidation (17).

Once diagnosed, triazole antifungal drugs are the first-line of treatment in diagnosed IA, although three different guidelines give slightly different recommendations (20,29,30). At present, Voriconazole is the gold standard of treatment after proving superiority over Amphotericin B deoxycholate, which has been the only treatment option for quite some time (33). However, in a 2016 study including 527 adult patients ISA proved non-inferiority to Voriconazole and, in addition, it was better tolerated with less hepatic, visual and cutaneous adverse effects, therefore representing an alternative in the first-line treatment of IA (1). Combination therapy of Voriconazole and Anidulafungin did not improve outcome compared to Voriconazole monotherapy and is generally not recommended, but might be useful in certain clinical settings, such as therapy refractory disease (34).

Given the known risk groups and outcome, precautionary measures are appropriate to protect patients from IA. In patients with neutropenia associated with myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML), Posaconazole is the preferred agent for IA

prophylaxis (22,35). Also, it is effective as prophylaxis in recipients of allogeneic hematopoietic stem-cell transplants with severe graft-versus-host-disease (36).

1.1.2 Mucormycosis

Mucormycosis is an infection caused by a group of ubiquitous, filamentous molds of the order Mucorales. Several genera cause invasive disease including *Rhizopus spp.*, *Mucor spp.*, *Rhizomucor spp*., and *Lichtheimia spp.* with *Rhizopus* being the cause in the majority of cases, followed by *Mucor* and *Lichtheimia* (37). These pathogens are likewise regarded as opportunistic. Infections with molds of the Mucorales order are associated with high rates of mortality.

Even though the incidence of mucormycosis is thought to be rising (38,39), the disease is sporadic and mucormycosis is designated as an orphan disease (40). As with many medical conditions, there are geographical differences in the incidence of mucormycosis which can also be associated with different underlying diseases in different regions. While the incidence in developed countries is reported to be as low as 0.1 to 0.2 per 100.000 persons and most of the patients affected by mucormycosis suffer from hematological malignancy (19), there is a higher incidence in developing countries e.g., India, where the incidence is around 14 per 100.000 population. The most common underlying diseases in India comprise first and foremost uncontrolled Diabetes mellitus and cutaneous infestations related to trauma (41).

Associated risk factors for mucormycosis include hematological malignancies with neutropenia, hematopoietic stem cell transplantation, SOT, immunosuppression, chemotherapy, autoimmune or rheumatic disorder, AIDS, peritoneal dialysis, iron overload status, malnutrition, trauma, burns, and prior therapy with Voriconazole (42,43).

The low overall incidence of mucormycosis limits the possibility of conducting large randomized-controlled trials. Therefore, most of the available data is based on case reports, case series, and multinational registries.

An infection with mucorales results most commonly from inhalation of fungal sporangiospores that have been released into the air. Another way of infection results from direct inoculation of organisms into disrupted skin or gastrointestinal tract mucosa (12). Dependent on the route of infection and underlying disease, the clinical manifestation varies

from rhino-orbito-cerebral, pulmonary, cutaneous and soft tissue, gastrointestinal to disseminated infections (37). Patients with impaired immune systems typically develop pulmonary mucormycosis, while diabetic patients more often suffer from a rhino-orbital form of the disease (43).

Radiologically multiple (>10) nodules, pleural effusion, and a reverse halo sign are commonly found in CT scans of patients with mucormycosis (44,45).

Upon suspected diagnosis of mucormycosis a patient should be immediately referred to a facility with the highest care. Treatment strategies for multimodal management of mucormycosis include early administration of antifungal drugs, optimizing predisposing underlying factors whenever possible, and rapid removal of all infected tissue (30,46). Furthermore, surgical debridement with clean margins should be undertaken to control disease and acquire tissue specimens for histopathology microbiological diagnostics (46). Surgical treatment is preferably initiated early, including surgeons in the management team and in the decision-making at the time of diagnosis (46).

Early diagnosis and prompt initiation of therapy are emphasized by a study that showed a nearly two-fold increase in mortality when the start of active antifungal treatment is being delayed (47). A lipid formulation of Amphotericin B is the first-line of antifungal treatment recommended in current guidelines. The suggested dose for liposomal AmB (lipAmpB) is from 5mg/kg/d to 10 mg/kg/d. A French study group treated patients with 10mg/kg/d in combination with appropriate surgery and the overall response rate was 36% at week 4 and 45% at week 12. In this study, as many as 40% developed renal impairment following therapy (48). ISA is recommended for the use in mucormycosis when preexisting renal impairment is present (46).

In the setting of graft-versus-host disease (GVHD) or neutropenic patients, primary prophylaxis is recommended with Posaconazole extended-release tablets to prevent mucormycosis (49).

Perspective in patients with mucormycosis is critical as all-cause mortality ranges from 40% up to 80% in patients with disseminated disease involving the CNS (50).

1.2 Antifungal therapy

Essentially, there are three categories of antifungals in use for IFD: Azoles, Echinocandins, and Polyenes. Next to these major categories, allylamines (Terbinafine) and Flucytosine are also available. Most antifungal drugs target ergosterol, which is a characteristic component of the fungal cell membrane. This mechanism of action disrupts fungal cell integrity and subsequently leads to cell death. In general, the choice of antifungals is limited (51). Consideration should also be given to concomitant non-drug strategies such as surgical management e.g., in case of deep-tissue infection (20,30).

1.2.1 Polyene Antifungals

Polyene antifungals have been isolated from culture filtrates of *Streptomyces spp*. since about 1955. The only available drug, which can be admitted intravenously is Amphotericin B (AmB). AmB was the first drug in use against IFD, and since it was introduced, new lipid-based formulations have been introduced to reduce toxicity. AmB has a wide spectrum of activity: *Cryptococcus neoformans*, most *Candida spp*., *Aspergillus spp*., and agents of mucormycosis. Fungal species resistant to AmB include: *Candida lusitaniae*, *Aspergillus terreus*, *Trichosporon spp*., *Geotrichum spp*., and *Scedosporium spp* (52). The fungistatic and fungicidal activity results from AmB binding to the fungal membrane, inducing a modification of membrane permeability leading to a higher efflux of electrolytes, and other cytoplasmatic, fungal cell components (51). Toxicity clearly limits the use of AmB. While lipid formulations have demonstrated better tolerability, nephrotoxicity remains an important side effect and AmB is only to be used if the newer lipAmB formulation is not available (46).

1.2.2 Triazoles

Triazole antifungal agents are widely used in prophylaxis and treatment of IFD. The more recently introduced triazoles Voriconazole, Posaconazole and ISA, have an extended spectrum compared to Fluconazole and Itraconazole. Azoles inhibit the synthesis of ergosterol via the fungal Cytochrome-P-450 enzyme C-14 alpha-demethylase. This mechanism of action leads to fungistatic activity in a wide range of fungi, including yeasts, filamentous fungi, dermatophytes, and dimorphic fungi (51). A disadvantage commonly encountered with Triazole antifungal agents is their large potential for drug-drug interactions, especially with drugs metabolized by the CYP system. TDM is frequently indicated in using Triazoles to manage the impact of drug-drug interactions and to avoid inadequate exposure (52).

1.2.3 Echinocandins

Echinocandins are cyclic lipopeptides inhibiting the 1,3-β-D-glucan-synthase, which provides 1,3-β-D-glucan as an important component to the fungal cell wall. Three substances are available: Caspofungin, Anidulafungin, and Micafungin. Echinocandins exhibit high fungicidal activity against yeasts but also showed a wide spectrum of *in vitro* activity.

1.2.4 Terbinafine and Flucytosine

Terbinafine inhibits early steps of ergosterol synthesis through blocking of squalene epoxidase. The resulting intracellular accumulation of squalene and depletion of ergosterol leads to its fungicidal activity. Terbinafine shows activity against dermatophytes, yeasts, some molds and some dimorphic fungi. It has been used as monotherapy or in combination with other antifungals in disseminated *Fusarium*, *Scedosporium*, or *Phaeohypomycetes* infections (52).

Flucytosine is converted into the antimetabolite 5-fluorouracil in fungal cells interfering with the synthesis of nucleic acids. Nowadays, Flucytosine is not being used frequently in clinical practice (51).

1.3 Isavuconazole

ISA, the active moiety of the water-soluble prodrug isavuconazonium sulfate, is a triazole antifungal agent used to treat invasive fungal infections caused by a variety of entities. ISA proved excellent activity against *Aspergillus spp*. Furthermore, it demonstrated very good activity against filamentous fungi of the Mucorales order (53). In animal models, ISA not only showed potency against *Candida spp*.(54), but also in *Cryptococcus spp*.(55). Moreover, ISA presented activity against other yeasts such as *Trichosporon spp*. (56). The broad spectrum of activity also includes *Coccidioides*, *Histoplasma* and *Paracoccidoides* (57).

Marketed under the tradename of Cresemba®, ISA has been approved in 50 countries worldwide and is available in 45 countries, including the United States (US), most European Union (EU) member states, and a few others countries worldwide (58).

ISA is either administrated intravenously or orally and is either way started with a loading dose of 200mg of ISA every 8 hours for the first 48 hours. 200mg are equivalent to 372mg of isavuconazonium sulfate, supplied in a single-dose vial as a sterile white to yellow powder

or as hard capsule containing 100 mg of ISA, equivalent to 186mg of isavuconazonium sulfate. Subsequently, the maintenance dose is 200mg ISA once daily given as a 1-hour infusion or orally with or without food, starting 12-24 hours after the last loading dose (59).

Table 3. Labeled EMA indications for the use of Isavuconazole (59)

In adults for the treatment of • Invasive Aspergillosis • Mucormycosis in patients for whom amphotericin B is inappropriate

IA and mucormycosis are considered to be rare diseases, and therefore ISA was designated an orphan medicine for aspergillosis and for mucormycosis (3).

1.3.1 Pharmacologic characteristics

Triazole antifungals express their fungicidal activity by blocking the synthesis of ergosterol, a key component of the fungal cell membrane. ISA intercepts ergosterol biosynthesis by inhibiting cytochrome P450 dependent 14α-lanosterol demethylation, an essential component of ergosterol production. This alteration in the fungal membrane induces cell death. While this mechanism is similar to existing triazole agents such as itraconazole, voriconazole, and posaconazole, ISAs structure was altered with a sidearm of a [N-(3-acetoxypropyl)-N-methylamino]- carboxymethyl group, which is discussed to be conferring activity against pathogens resistant to other azoles (60).

The water-soluble prodrug isavuconazonium sulfate was developed to overcome the nephrotoxic potential of cyclodextrin, which is used in the intravenous formulation of voriconazole and itraconazole. This is no longer necessary in ISA to facilitate solubility (60). After administration of the prodrug, plasma esterases convert isavuconazonium sulfate nearly entirely (>99%) into the active moiety, whereas the water-soluble inactive cleavage products in the plasma can only be measured in minimal amounts and do not seem to be clinically relevant (61). Isavuconazonium sulfate is also being transformed via nonenzymatic (chemical) cleavage of the prodrug in the gut (62).

Advantageously, after both oral and intravenous administration, ISA exhibits excellent bioavailability, with approximately 98% of the active drug passing into circulation, regardless of the chosen form of administration (see Table 4). Maximum plasma concentrations in healthy individuals are reached after 2-3 hours of oral administration and

after 1 hour of intravenous administration (61,63). Oral bioavailability is neither affected by gastrointestinal pH altering drugs nor by food intake (64). ISA demonstrated a large volume of distribution and low clearance. The long terminal half-life elimination was assessed to be 100h (62,63). Linear pharmacokinetics were shown in population pharmacokinetics (PK) models (65).

The protein-bound fraction of ISA in plasma accounts for >99%, with Albumin being the predominant binding protein. Indicated by a large volume of distribution ISAs tissue penetration is generally considered good including liver, lungs, eyes, kidneys, bone, nasal mucosa, and brain (63). Tissue penetration is also supported by inherent physicochemical properties that favor membrane permeability such as low molecular weight (<500g/mol), polarity, and lipophilicity. Regarding penetration of sanctuary sites (brain, testes) ISA is not considered a substrate of efflux pumps e.g. P-glycoprotein. Thus, ISAs penetration of sanctuary sites is thought to be good.

A murine model assessing brain tissue concentrations directly showed a ratio of ISA brain /plasma concentrations of 1.8:1 similar to voriconazole (2:1). Voriconazole represents the first-line treatment of cerebral IA (66). ISA was stated as an option in the treatment of fungal infections involving the CNS (67).

The same model indicated that steady-state concentrations might only be reached by day 14 in some tissues. Whereas the highest maximum concentrations were found in bile, liver, adrenal glands, adrenal cortex, and brown fat tissue, ISA concentration values were lowest in bone, eye lens, eye, seminal vesicles, and olfactory bulb. These values were obtained via quantitative whole-body autoradiography following oral administration of radiolabeled isavuconazonum sulfate to rats (66).

Intersubject variability of maximum concentration (C_{max}) and area under the curve 24hours after the first dose were low to moderate (63). The assessed intra-subject variability of ISA trough levels was low in the SECURE trial (1).

Table 4. Key pharmacokinetics of Isavuconazole (14)

Characteristics	Isavuconazole
Dosage	200mg q8 hours day 1+2; 200mg q 24 hours maintenance
Resorption PO/IV	PO = IV
Oral intake with/without food	No Influence
Half-life, hours	100 h
Oral bioavailability	98%
Linear pharmacokinetics	Yes
Central nervous system penetration	High (animal model)
Renal excretion	<1%
Metabolism	CYP3A4/5
Exposure-response relationship	No
Intra-patient variability	Low
Inter-patient variability	Low to middle

1.3.2 Metabolism and Excretion

ISA is metabolized in the liver via cytochrome-P-450 (CYP) isoenzymes, particularly CYP3A4 and CYP3A5. Metabolism and excretion were studied in healthy volunteers first receiving single-ascending doses of the prospective new antifungal BAL4815 and its prodrug BAL8557, followed by studies with multiple doses. Lately, studies with radio-labeled isavuconazonium sulfate contributed to a better understanding of metabolic pathways (61,63,66).

A mean of 46,1% of the total radioactive dose, administered orally, was recovered in the feces, and 45,5% was recovered in the urine, although renal excretion of ISA itself was <1% of the given dose. The major part of urinary excretion was an inactive oxidative carbamate metabolite (62). This is consistent with previous results of very low urinary excretion of ISA. Therefore it is postulated that no dose adjustments have to be made in mild, moderate, or severe renal impairment (68). ISA is presumed not to be dialyzable (69).

The influence of mild or moderate hepatic impairment onto ISAs metabolism was studied in a population pharmacokinetics model (PPK), which itself was based on studies including patients with hepatic impairment due to alcoholic liver cirrhosis and due to hepatitis B/C. Safety data and trough concentrations acquired in this setting indicate that there is no need

for dose adjustments in patients with mild or moderate hepatic impairment as the increase in trough concentration is less then two-fold in these patients (70).

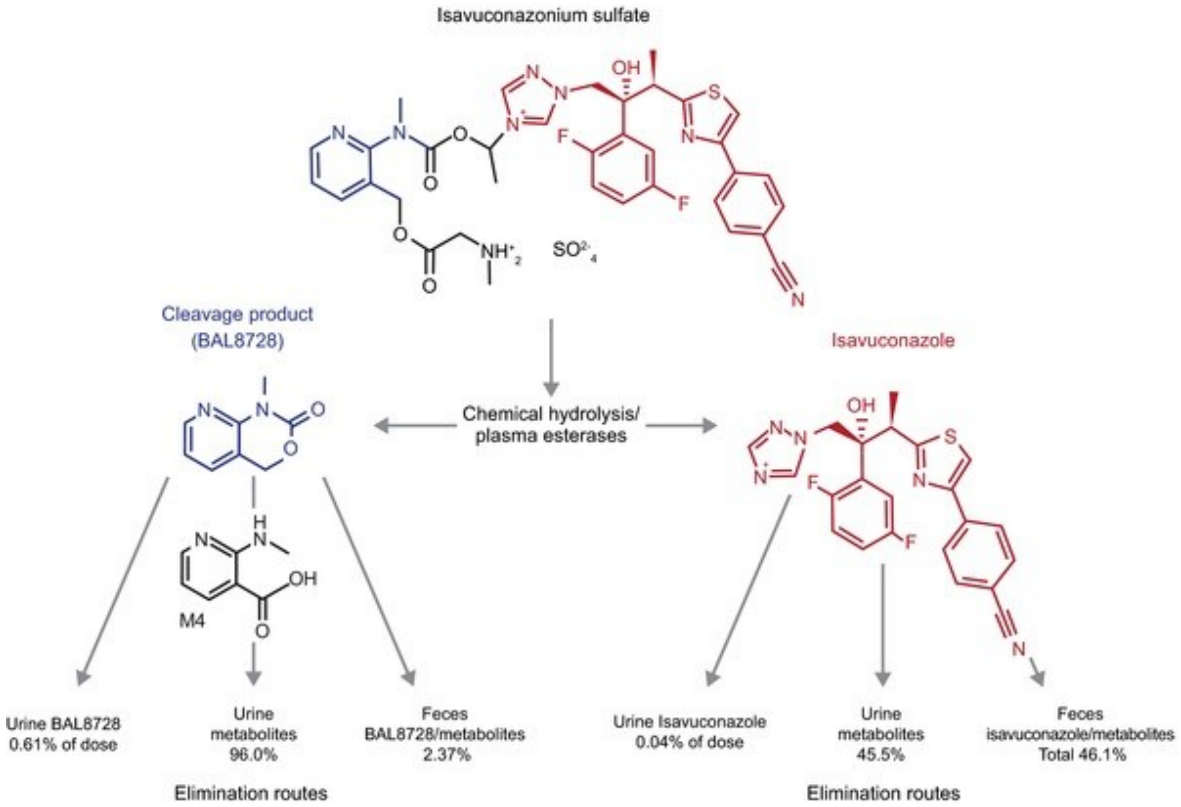

Figure 2. Metabolic pathways of isavuconazole. Townsend et al. postulated metabolic pathways for isavuconazonium sulfate after administering single doses of radioactive-labeled isavuconazonium sulfate in healthy individuals. (62) The figure shows the elimination routes of major metabolites.

Asian race has been discussed as a parameter that was previously associated with reduced clearance of ISA. Desai et al. described a lowered clearance of 36% in Asians in comparison to Caucasions, which resulted in a 40% difference in exposure (65).

1.3.3 Adverse effects

Overall, ISA has been described to be safe and well-tolerated. Single-ascending dose and multiple-dose studies undertaken in evaluating pharmacokinetics and safety of ISA in healthy volunteers reported only mild or moderate adverse events except for one severe rhinitis event, which was not related to trial medication. Frequent side effects included upper respiratory symptoms, headache, and mild gastrointestinal symptoms, including abdominal pain nausea, and diarrhea (61,63).

Serious adverse effects were found in three patients of a randomized, double-blind, multicenter phase II trial that evaluated the safety and efficacy of three different dosing regimens of ISA compared with Fluconazole in patients with uncomplicated esophageal

candidiasis, namely atrioventricular block, tuberculosis pleurisy, and a moderate to severe increase in liver enzymes. The intent-to-treat (ITT) population consisted of 121 patients in the different groups receiving ISA. Overall, in this study four patients discontinued treatment with ISA due to treatment-emergent adverse events (TEAE), whereas two were considered unrelated to the study drug (71).

Permanent drug discontinuation occurred in 21 (8%) of the patients in the SECURE trial due to drug-related adverse events. Nearly all of the patients (247/257, 96%) in the ISA safety population had at least one TEAE. The most common adverse events were nausea, vomiting, diarrhea, pyrexia, and hypokalemia. (Table 5) The ISA-treated patients in the SECURE study had a significantly lower frequency of hepatobiliary disorders (9 vs. 16%), eye disorders (15% vs. 27%), and skin or subcutaneous tissue disorders compared to the Voriconazole group. There was no significant difference in serious treatment-emergent adverse events between the study groups. Laboratory parameters and ECG were also reviewed and showed no clinically relevant trends (1).

In the VITAL study, similar rates of patients suffered from TEAEs as in the SECURE study of IA. 35 out of 37 patients being treated with ISA reported at least one adverse event in the course of treatment, with the most common being very similar to the SECURE study outcomes. Adverse events led to study drug discontinuation in six patients and were attributed to the following conditions: relapse or progression of malignant disease (two patients), acute liver injury (two patients), *Escherichia coli* bacteremia (one patient), and nausea (one patient). Elevation of alanine transaminase, aspartate aminotransferase, or other hepatic enzymes were seen in less than 10% of the patients (2).

A rise in liver transaminases greater than ten times the upper limit occurred in 1.2% of patients in the SECURE trial, whereas rises above three times the upper limit were reported in 4.4% out of 257 patients at the end of study treatment (1).

ISA shortens the QTc interval dose-related, in contrast to the common QT interval prolongation effect of other azole antifungals. The actual risk of drug induced shortening of the QTc-interval and its mechanism in administering ISA is not yet clear. However, ISA is contraindicated for patients with familial short-QT-syndrome, because of known complications resulting from a short QTc time. Special precautions should be taken when

combining ISA with other drugs that reduce the QTc interval, because of unknown additive effects (72).

Table 5. Frequently reported treatment-emergent adverse events in ISA-treated patients; Data from a total of 403 patients in two clinical trials (73)

Nausea (26%)
Vomiting (25%)
Diarrhea (22%)
Headache (17%)
Elevated liver chemistry tests (16%)
Hypokalemia (14%)
Constipation (13%)
Dyspnea (12%)
Cough (12%)
Peripheral edema (11%)
Back pain (10%)

1.3.4 Drug-drug interactions

The potential of drug to drug interactions in ISA and other triazole antimycotics results from their interaction with the CYP system. The human CYP system is a large system of oxygenases located in the endoplasmic reticulum of liver cells, with their main function being the biotransformation. Biotransformation is part of the metabolization of drugs. Three subgroups of the CYPs are predominantly relevant in the process, with their most important enzyme CYP3A4 being involved in as many as 40-45% of all biotransformations (74). ISA is a sensitive substrate and a moderate inhibitor of CYP3A4 (75).

The simultaneous administration of ISA and drugs acting as strong CYP3A4 inhibitors is contraindicated. Furthermore, the coadministration of ISA with strong CYP3A4 inducers is contraindicated (73).

The effects of concomitant medication interacting with the CYP system was studied in clinical trials examining the comedication of ISA with either rifampin (strong CYP3A4 inducer), ketoconazole (strong CYP3A4 inhibitor and substrate), midazolam (CYP3A4 substrate, or ethinyl estradiol/norethindrone (CYP3A4 substrate). Coadministration of rifampin with ISA confirmed the well-established connection between triazole antifungals

and strong inducers of the enzyme CYP3A4 (76) resulting in reduced ISA levels. During coadministration with ketoconazole, ISA levels rose significantly, which could also be seen during coadministration with another strong CYP3A4 inhibitor, lopinavir/ritonavir (77).

A report evaluated the pharmacokinetic interactions in several phase 1 studies between ISA and the immunosuppressants cyclosporine, mycopheonolic acid (MMF), prednisolone, sirolimus, and tacrolimus in healthy adults. The authors conclude that levels of the immunosuppressants cyclosporine, sirolimus, and tacrolimus potentially increases when ISA is being coadministered and therefore recommend TDM to assess the necessity of dose adjustments of the immunosuppressants (78). In a comparative evaluation of ISA, voriconazole, and posaconazole, it was shown that dose reduction in the case of concomitant immunosuppressant therapy was statistically lowest for ISA (79).

ISA has also been discussed to be an inhibitor of phase II enzyme uridine diphosphate glucuronosyltransferase (UGT), which is of interest when patients receive MMF and additional treatment with ISA. myophenolic acid, the active agent of MMF, AUC was increased up to 35% in the presence of ISA administration (78).

Table 6. Plasma pharmacokinetics of ISA in the presence and absence of rifampin and ketoconazole. Schematically illustrating a study for drug-drug interactions. (75)

	ISA PK (± Rifampin)		ISA PK (± Ketoconazole)	
Parameter	ISA Alone (n=25)	ISA + Rifampin (n=24)	ISA Alone (n=12)	ISA + Ketoconazole (n=12)
AUC0-∞ ,h*µg/ml	Base value	⇓⇓⇓	Base value	⇑⇑⇑
Cmax, µg/ml	Base value	⇓⇓⇓	Base value	⇑
T 1/2, hours	Base value	⇓⇓⇓	Base value	⇑⇑⇑

In more detail, ISA is a mild inducer of CYP2B6, but does not interact with CYP1A2, CYP2C8, YP2D6 or CYP2C19.(80) Coadministration of ISA and Warfarin, a CYP2C9 substrate, did not affect the pharmacodynamics of the coumarine.(81)

Table 7. Agents of potential major or life-threatening interactions: concomitant use is contraindicated (82)

Anticonvulsants

- Carbamazepine
- Phenobarbital
- Phenytoin

Antibacterials

- Rifampin
- Rifabutin
- Nafcillin

Antifungals

- Ketoconazole

Herbal medicines

- St John's wort

Antiretroviral agents

- Efavirenz
- Etravirine
- High-dose ritonavir

1.3.5 Current guideline recommendations for ISA

Recommendations for the clinical use of ISA have been made in various guidelines. The following section outlines the different expert panels, and their recommendations of ISAs use in clinical practice.

1.3.5.1 Infectious Diseases Society of America (IDSA)

In their guideline for the diagnosis and management of aspergillosis published in 2016, triazole antifungal agents are the treatment of choice in IA in most patients. Serum trough levels should be obtained for most azole antifungal agents, while further data is required to assess the requirement of TDM for ISA. Drugs inducing CYP3A4 should also be closely monitored as to their high potential of interaction.

ISA is recommended as an alternative to voriconazole for the primary treatment of IA. (strong recommendation; moderate-quality evidence) based on the outcomes of the SECURE study. Even though ISA has not been evaluated in a salvage setting, authors of the guideline consider its use justifiable if it has not been used in primary therapy (29).

1.3.5.2 ECIL-6

The European Conference on Infections in Leukemia (ECIL) last published recommendations for treating invasive candidiasis, aspergillosis and mucormycosis in leukemia and hematopoietic stem cell transplant patients in its sixth update (ECIL-6) in 2016. ISA is recommended as first-line treatment in IA (strong recommendation; good-quality evidence), thus putting ISA on the same level with voriconazole. This recommendation is based on the SECURE trial.

In the management of mucormycosis, ISA is only mentioned as a potent in-vitro agent, but no recommendation in favor of its clinical use is made (30).

1.3.5.3 Global Guideline for the diagnosis and management of mucormycosis

In an effort of the European Confederation of Medical Mycology (ECMM), the Global guideline for the diagnosis and management of mucormycosis was published late in 2019 as part of the "One world One Guideline" initiative. The licensing of ISA, among other developments, is recognized as a fundamental change in mucormycosis management. In Europe ISA is licensed for salvage treatment of mucormycosis. However, ISA is recommended for the first-line treatment of mucormycosis by the guideline consortium with moderate strength. As salvage treatment, ISA is strongly supported.

With less nephrotoxicity, ISAs potential for the use in cases of renal failure has been shown. In patients with poor prognosis, ISA may also be added to a first-line therapy of liposomal amphotericin B (46).

1.4 Therapeutic drug monitoring (TDM)

TDM is performed to measure exact blood levels of a certain administered drug to evaluate the individuals' exposure to the drug. Practically, this requires collecting a blood sample at a certain time relative to prior application of the drug.

TDM proves to be of use in monitoring drugs with the following key characteristics:

- Significant variability in pharmacokinetics
- Narrow therapeutic index
- Predefined therapeutic range of effective concentration

Besides, there has to be the possibility of quantifying the drug with an adequate mean, e.g., an assay that is specific, accurate, and precise.

The purpose of TDM encompasses enhancing therapeutic efficacy, evaluating therapeutic failures due to suboptimal drug exposure, and to minimize toxicities due to overexposure. Moreover, adequate drug exposure is important to ensure the reduction of resistance development.

Besides its utility, TDM it is an additional procedure in the therapeutic setting that consumes medical staff's working time, expertise, and last but not least financial means.

1.4.1 Therapeutic Drug monitoring of Triazole antifungals and ISA

Current guidelines recommend routine TDM in triazole-based therapy with a moderate amount of data supporting its use for itraconazole, voriconazole, and posaconazole suspension (20,29,30). In contrast, there is no recommendation to routinely measure ISA trough levels as to its advantageous pharmacokinetic profile. Despite its preferable pharmacokinetics, ISA is still relatively new, and data regarding the need for TDM of ISA in special populations of patients is rare.

Plasma trough concentrations of ISA were measured in 283 cases of aspergillosis in the SECURE trial, where low intra-subject variability of those concentrations was found. The trough-level distribution (inter-patient variability) in the ISA group was found to be narrow, with ISA trough levels at day 14 of the trial ranging from 0.81 μg/mL to 9.95 μ/mL with a mean of 3.35 μg/mL (SD 1.81 μg/mL) (1).

ISA trough levels in real-world practice were compared to trough levels from clinical trials. Values obtained in clinical practice were statistically lower, but overall consistent with the results from three phase III studies. About 10% of the patients were identified with levels below 1μg/ml (83).

There is limited data about concentration thresholds in ISA. The exposure-response relationship of ISA has been studied in an analysis of the SECURE trial. No statistically significant relationship was found between ISA exposure and either efficacy or safety endpoints (84).

Clinical breakpoint concentrations have been defined by the European Committee on Antimicrobial Susceptibility Testing with 2mg/l has as the highest clinical breakpoint for ISA (85).

In mucormycosis, the therapeutic range for ISA is unknown. In the VITAL study which evaluated ISA for the treatment of mucormycosis median ISA trough plasma concentration was 3.32 µg/mL (Interquartile Range [IQR] 1.95-4.10, n=13) on day 7, 3.47 µg/mL (IQR 0.84-5.65, n=11) on day 14 and 4.19 µg/mL (IQR 3.04-5.70, n=18) on day 28.

In the management of patients with mucormycosis treated with ISA, routine TDM is not recommended, based on the same reasons as to the treatment in IA. However, serum drug concentrations could be of use in various clinical situations, e.g., suspected treatment failure, drug interactions, suspected toxicity or intolerance, obesity or after switching IV to oral therapy (46).

In selected patients, TDM for ISA might offer clinical insights in certain situations, but data to support its use is still scarce. The IDSA provides a list of clinical scenarios where TDM is useful in their guideline for the management of IA (see Table 8.)

Table 8. Clinical Scenarios where TDM Is Useful in Treatment of Aspergillosis (29)

Clinical Scenario	Examples, Comment
Populations with increased pharmocokinetic variability	Impaired gastrointestinal function, pediatric patients, elderly patients, obese patients, critically ill patients
Changing pharmocokinetics	Intravenous-to-oral switch, changing GI function, changing hepatic or renal function, physiological instability
Interacting medications	CYP3A4 inducing agents, antiretroviral medications
Severe disease	Extensive infection, lesions contiguous with critical structures, CNS infection, multifocal or disseminated infection
Compliance	Longer-term consolidation therapy or secondary prophylaxis
Suspected breakthrough infection	Excluding inadequate antifungal exposure in the progression of fungal disease under treatment
Suspected drug toxicity	

1.5 Defining responses to Therapy and Study Outcomes in IFD

Standard definitions of IFD were proposed and have become common practice in clinical trials (27). Similarly, responses to therapy and study outcomes in clinical trials of IFD were defined to overcome challenges in the design of antifungal trials.

The different major entities of IFD compile specific criteria for successful treatment outcome. Still, it can be agreed on that global response requires survival and a positive effect on fungal disease. General response criteria are summarized in Table 9.

Table 9. Global responses to Antifungal Therapy (86)

Outcome, response	
Success	Failure
Complete response	Stable response
Partial response	Progression of fungal disease
Death	Death during the prespecified period of evaluation, regardless of attribution

Stabilization of fungal disease during periods of severe immunocompromise can under certain circumstances be equated with evidence of the efficacy of treatment. It may be a reasonable short-term therapeutic goal until immune recovery occurs (86).

In evaluating primary therapy outcomes in IA, six weeks proved to be a relevant measure as the minimum time of treatment before assessing outcome in IFD. For example, the SECURE trial defined all-cause mortality after 6 weeks as the primary outcome measure (1). Another analysis after twelve weeks, could represent a secondary outcome parameter. After 6 weeks of treatment, deaths can be increasingly attributed to causes other than the fungal infection (87). For non-*Aspergillus* invasive mold disease, it is also reasonable to extrapolate these time points (86). Especially early into the course of treatment, clinical, radiological, and mycological endpoints may conflict (88).

Specific criteria in assessing the outcome of antifungal therapy in patients with invasive mold disease have also been summarized in the guideline (86).
Treatment success comprises two categories. **Complete response** of antifungal treatment in a patient means survival and resolution of all attributable symptoms and signs of disease.

The radiological persistence of residual changes such as scars or postoperative lesions can be equated with a complete radiological response if there are no florid lesions radiologically detectable. If it is feasible to sample the infection site, a documented clearance would be another criterion for such complete response.

The second positive outcome category, a **partial response,** is defined as the survival and at least the improvement of attributable symptoms and signs of disease. The improvement should be seen in radiologic assessments to reduce lesions of at least 25% in diameter. If there is a 0%-25% decrease in radiological lesions' diameter, it is called radiological stabilization and can be argued to be a partial response. If the biopsy of an infected site shows no evidence of hyphae and negative culture results, this should be equated partial response when the radiological improvement is only stable (0%-25% decrease in diameter).

Treatment failure can be divided into three categories. A **stable response** in antifungal treatment means the survival of the patient with minor or no improvement in attributable symptoms and signs of disease as well as the radiological stabilization. If sampling of infected sites results in persistent isolation of mold or histological presence of invasive hyphae it is considered a stable response in treatment failure.

The worsening of clinical symptoms or signs of disease in addition to new sites of infection and deterioration of preexisting radiological lesions or the persistence of isolation mold species from infected sites is a **progression of fungal disease**.

A patients **death** in the prespecified period of evaluation regardless of attribution, is interpreted as treatment failure (86).

Overall, it is difficult to evaluate responses in IA and other invasive mold diseases. A lack of specific physical signs such as the low occurrence of fever in the highly immunocompromised represents challenges in the clinical assessment of outcome criteria (89). Clinical manifestations may even be misleading, e.g., hemoptysis is more often seen after neutrophil recovery, which itself may indicate a favorable finding (90). Further, radiological improvements usually come up after prior deterioration of the typical signs of IFD (88). For example, pulmonary cavitation may also be seen in the context of neutrophil recovery and should, therefore, not be put on the same level as fungal disease progression

(86). Accordingly, a follow-up chest CT-scan is recommended after a minimum of two weeks of treatment by America's infectious diseases society (IDSA) (29).

Repeated sampling of infected sites (e.g. lung-biopsy) is not an option in most cases because of its impracticability.

In patients with hematologic malignancy or hematopoietic stem cell transplantation (HSCT) serial measuring of serum Galactomannan (GM) can be useful in assessing therapeutic response when baseline GM is elevated (29).

One perspective for the future of defining responses might be in developing a surrogate marker for treatment response. This could be represented by a non-culture-based laboratory assay (e.g. PCR) (86).

1.6 The Clinical Problem

In the case of IFD, treatment options are limited to a small number of antifungal drugs available. ISA is the latest addition to the triazole antifungals and has proven efficacy in two large phase III trials in the treatment of IA and mucormycosis. The data gathered in these trials provide basic evidence for ISA use but does not offer lots of information about real-world use. Against this background laid out briefly, the aim of this work was to present data gathered in the clinical use of ISA to reduce shortcomings of the previous studies and build a bridge to daily clinical practice.

2 Materials and Methods

2.1 Study population

32 patients undergoing overall 33 treatment courses with ISA were identified and included in this investigation. These patients were recruited from the following four centers in the Southern and Eastern part of Austria: the Medical University of Graz, the Landeskrankenhaus (LKH) Graz II, LKH Hartberg, and the Klinikum Baden. Resulting in a mixed patient cohort, this group of patients reflects a real-life setting, including patients with non-malignant disease and extracorporeal treatment.

The following inclusion criteria were applied:

- 18 years or older
- received treatment with ISA between November 2016 and October 2019 as part of the routine clinical care

2.2 Study design

The observational national multicenter cohort study investigated patients undergoing treatment with ISA from November 2016 to April 2019. Patients medical records were reviewed individually by using a standardized data collection template to collect demographic information and clinical data, mycological laboratory test results, ISA trough plasma concentrations, as well as ISA formulation, dosing information, termination of ISA treatment as well as other antifungal therapy, clinical response, serious adverse events, and breakthrough Infections.

In accordance with the local drug committee, the use of ISA was allowed in patients with possible/probable/proven IA using revised EORTC MSG criteria and/or Blot criteria in case of invasive aspergillosis in critically ill patients (27,28). When fulfilling the criteria of probable/proven mucormycosis for whom liposomal lipAmpB was not appropriate, patients were eligible for ISA treatment. These patients either had adverse events under lipAmpB treatment, a lack of clinical response, or there were contraindications to lipAmpB in the first place. Despite, IA and mucormycosis, ISA was administered in patients with other probable/proven invasive fungal infections based on antifungal susceptibility testing favoring ISA over alternative antifungals. Treatment consisted of the standard dosing regimen of ISA.

Treatment outcomes were classified according to EORTC/MSG criteria (86). Due to ISA's different indications in the mixed patient cohort, assessment of efficacy was not carried out at prespecified timepoints but throughout the treatment followed by an observational time period. The observation interval was extended up to two months after the end of treatment and, if necessary radiological studies were also reevaluated. Breakthrough infections under ISA were assessed, as stated in the revised ECMM/MSG criteria (91).

Emergent side effects like QT abnormalities, neutropenia and hepatotoxicity, were observed by electrocardiograms (ECG) and laboratory assessment of blood cell count and liver enzymes such as alanine aminotransferase (AST), aspartate-aminotransferase (ALT), and alkaline phosphatase (AP), respectively. Headache, abdominal pain, nausea, vomiting, and other treatment-emergent side effects were assessed reviewing the patient's documents, drawn up during their hospitalization or at outpatient visits, for mentioned complaints.

To evaluate the duration of drug intake and trough concentrations throughout treatment, the first day of administering ISA was declared day 0. No standard TDM algorithm was available by current literature (84,92). Hence, plasma samples for ISA TDM were obtained as ordered by the treating physicians and adjusted to, e.g., scheduled outpatient visits. To acquire proper trough concentrations of ISA, the samples for quantification were obtained in the morning immediately before the following dose of ISA infusion or intake when ordered by the treating physician.

For the measurement of ISA plasma concentrations, electrospray ionization tandem mass spectrometry on a Voyager TSQ Quantum triple quadrupole instrument equipped with an Ultimate 3000 chromatography system (Thermo Instruments, San Jose, California, USA) was used. To guarantee the standard of care, the laboratory investigating ISA plasma concentrations participated in international ISA round robin tests (93).

The local ethics committee approved the study. Medical University Graz, Austria (protocol number 29-444 ex 16/17).

2.3 Statistical analyses

The statistical analyses were performed using R version 3.5.1. Continuous data (i.e., ISA plasma concentrations) are shown as medians and interquartile ranges (IQR). Data regarding single patients were summarized by calculating the median (alternatively maximum) of ISA

levels, e.g., in patients with more than one single measurement, the median and maximum levels of ISA were calculated to make sure that each patient is represented by one single summary measurement (median or maximum) and standard statistical tests can be applied. For inter-group comparisons, the median was used as the main variable, while the maximum value was used for sensitivity analysis. Measurements of patients assigned to specified groups (e.g., with or without extracorporeal treatments) were considered independent. Analyses of continuous independent data were performed by Wilcoxon-rank-sum test. A p-value of <0.05 was considered significant.

3 Results

3.1 Study Cohort

ISA was administered to 32 patients overall with mixed underlying disease. Proven invasive fungal disease was found in fourteen of 32 patients (44%), probable invasive fungal disease in nine patients (28%), and nine patients (28%) had possible invasive fungal disease (see table 10). Two courses of ISA were evaluated in one and the same patient having probable invasive fungal disease on both occasions. General patient demographics, including frequency of underlying diseases, are presented in detail in table 11.

A total of 23 (71%) male patients and 9 (29%) female patients with a median age of 59 years (range 24 to 85 years, interquartile range [IQR] 46-69) and with a median body mass index (BMI) of 24.7 kg/m2 (range 18.5-39.6, IQR 23.1-29.8) were investigated. One patient received two courses of ISA. Most commonly, the patients had a hematological disease as the underlying condition (45%), whereas other patients suffered from various underlying diseases (see table 11). Renal replacement therapy (RRT; continuous hemodialysis, continuous hemofiltration, or a combination of both) was present in 11 out of 32 patients. Three of these patients underwent therapy with extracorporeal membrane oxygenation (ECMO). One patient additionally had Cytosorb® adsorber therapy (CytoSorbents Europe, Berlin, Germany).

Table 10. Diagnoses of Fungal Disease according to the EORTC Definitions of Fungal Disease

Proven, No. (%)	14 (42 %)
Probable, No. (%)	10 (30 %)
Possible, No. (%)	9 (27 %)

The duration of ISA intake ranged from one day to a maximum of 441 days, with a median of 45 days (IQR 16-106 days). In 15/33 (45%) of the ISA courses, the drug was administered intravenously and orally, switching from one formulation to another, while 12/33 (36%) received ISA only intravenously. 6 out of 33 (18%) treatment courses were solely carried out with the oral formulation of ISA. The Median duration of the intravenous formulation was 13 days (range 1-54 days, IQR 8-21), and the tablet was used for a median of 70 days (range 8-414 days, IQR 34-201).

Antifungal therapy or prophylaxis prior to treatment with ISA was given in a total of 20/33 (61%) treatment courses, with the majority of patients (13/20, 65%) being switched to ISA after encountering side effects due to preceding antifungals. Insufficient treatment success with prior antifungal therapy lead to a switch to ISA in 6/20 courses.

Table 11. Demographics of Isavuconazole (ISA) treated patients.

32 ISA patients	
Age, median (IQR), years	60 years (46-69)
Female sex, No. (%)	9 (28 %)
Weight, median (IQR), kg	75 (65-84)
Body Mass Index (BMI), median (IQR), kg/m²	24.6 (23.3 – 28.5)
<18.5: underweight, No. (%)	0 (0 %)
18.5 to <25: normal, No. (%)	19 (59 %)
25 to <30: overweight, No. (%)	7 (22 %)
≥30: obese, No. (%)	6 (19%)
Underlying diseases	
Hematological disease*	14 (44 %)
Solid cancer	2 (6 %)
Solid organ transplantation	4 (13 %)
Collagenosis/autoimmune diseases	2 (6 %)
Type 2 diabetes	2 (6 %)
Respiratory tract diseases	3 (9 %)
Bacterial infections	3 (9 %)
Trauma associated osteomyelitis	1 (3 %)
Coronary heart disease	1 (3%)

** 5 Patients with acute myeloid anemia, 1 patient with aplastic anemia, 2 patients with acute lymphatic leukemia, one patient with chronic lymphatic leukemia, 4 patients with lymphoma and 1 patient with hemophagocytic syndrome*

3.2 Isavuconazole Efficacy and Safety

A successful treatment outcome in the sense of complete response was assessed in 18/30 (60%) ISA treatment courses. Partial response accounted for 8/30 (27%) of the treatment courses, while a stable response occurred in 1/30 (3%). ISA levels were similar with no significant differences in these groups. Overall, retrospective assessment of outcome proved to be impossible in three cases because of different circumstances (one patient received only one dose of ISA, one patient with acute aortic valve avulsion, and one patient with secondary sclerosing cholangitis).

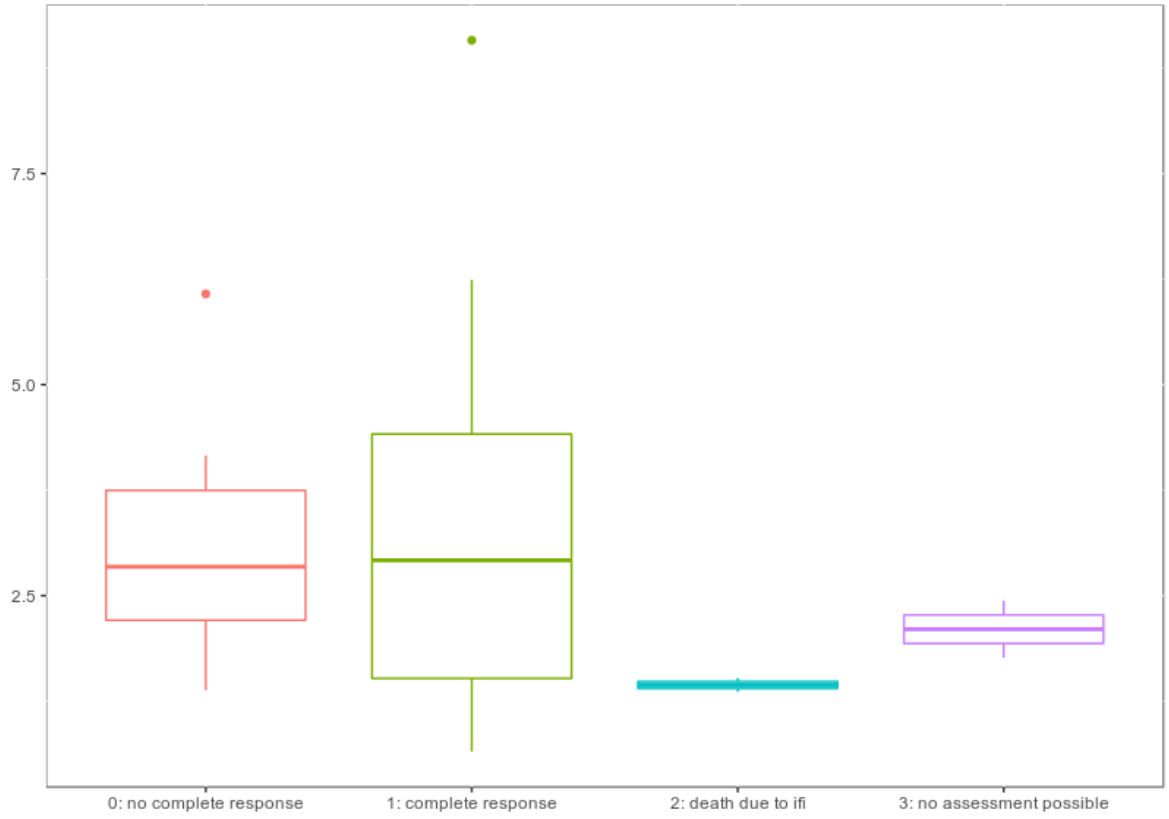

Figure 3. Boxplot Graph of Treatment Outcomes in Isavuconazole (ISA) treated patients with corresponding ISA Trough Levels

Fatality attributed to fungal disease in patients receiving ISA added up to three patients out of 32 (9%) patients. One patient with histopathological proven disseminated aspergillosis died after only receiving four days of ISA treatment with no plasma concentrations being measured. Another patient died on day 10 of treatment with possible invasive mold disease, although his radiological studies were highly indicative of invasive mold disease. ISA TDM showed plasma levels of 1.36µg/mL on day 3 of treatment (without RRT) and 1.69µg/mL on day 6 while on continuous hemodialysis. The third patient whose death was attributable to invasive fungal disease received treatment with ISA for six days before it was switched to lipAmpB plus voriconazole. Antifungal susceptibility testing of isolated *Fusarium solani* suggested a change of drug regimen, because minimal inhibitory concentrations (MIC) for

ISA were > 32µg/mL, while MIC for lipAmpB and Voriconazole was more favorable. This patient died 6 weeks after antifungal therapy was initiated. TDM, in this case, showed plasma concentrations of ISA of 1.51µg/mL on day 4, 1.32µg/mL on day 6, and two days after discontinuation of therapy with ISA 1.36µg/mL.

Adverse events were observed in six out of 33 (18%) of the ISA treatment courses, including one patient with a serious anaphylactic reaction (dyspnea and generalized erythema). The other adverse events encountered were one case of leucopenia (1.52G/L), two cases with elevated liver enzymes, one case with paresthesia, and one case with both elevated liver enzymes and erythema. Because all of the patients received concomitant medication ISAs role in the reported adverse events remains unclear. All patients recovered fully from adverse events.

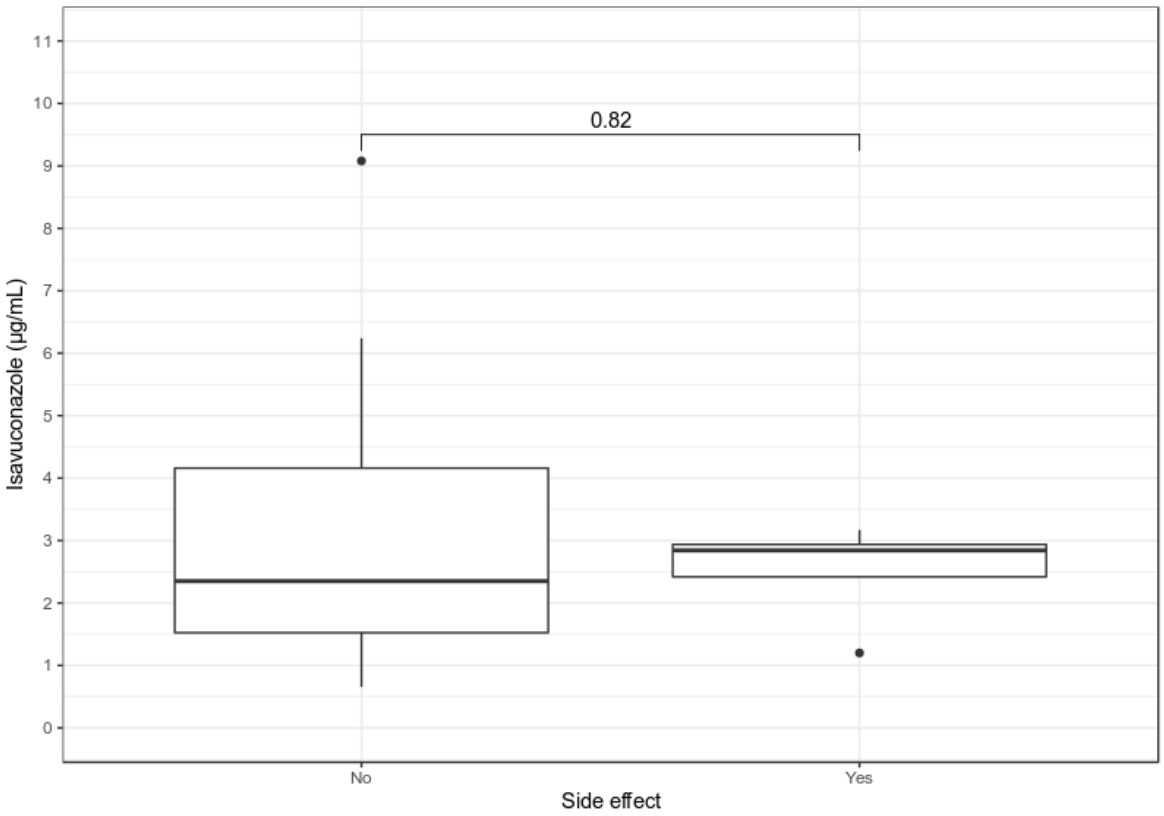

Figure 4. The Presence of a Treatment Emergent Side Effects with the Conrresponding Isavuconazole Trough Level. No threshold for drug-level associated toxicity was identified.

3.3 Isavuconazole TDM

Overall, 145 samples were collected to measure ISA plasma trough concentrations. After excluding five sampled values, due to incorrect timing of collection (collection of samples after drug intake in three instances, two samples were collected after the end of treatment), 140 ISA plasma concentrations from 29 courses of ISA treatment were analyzed. The

median of measurements per patient was three, with a range from 1 one to 18. Samples for TDM were taken after a median of 2.28 days (range 0.74-9.09; IQR 1.51-2.86) into the ISA treatment course.

The median plasma concentration was 2.35µg/mL (range 0.66 to 9.1µg/mL IQR 1.49-3.71). Excluding values obtained from patients with concomitant RRT or ECMO the group's median plasma concentration without RRT was 3.05µg/mL (range 1.38-9.1, IQR 1.93-4.35).

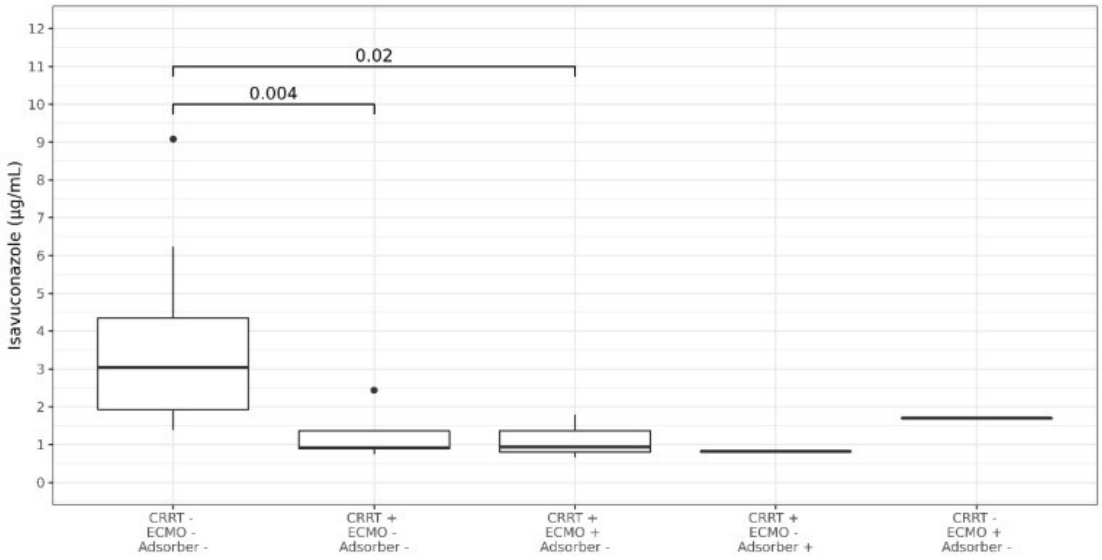

Figure 5. Boxplots showing median ISA trough plasma concentration in patients with and without extracorporeal treamtnes. CRRT = renal replacement therapy, ECMO = extracorporeal membrane oxygenation, adsorber = cytosorb adsorber. P-values shown above the brackets. (94)

Seven patients received RRT while being treated with ISA, and their median plasma concentration was 0.91µg/mL (range 0.66-2.44, IQR 0.82-1.36). Compared to the group without RRT, the plasma concentrations were overall lower in the period of treatment.

Four of seven patients in the group with RRT additionally received treatment with extracorporeal treatments (4 patients with ECMO, 1 patient with Cytosorb® adsorber therapy), and median ISA plasma concentration in this group was 0.91µg/mL (range 0.75-2.44, IQR 0.90-1.36). Two of the patients needing extracorporeal treatment with ECMO (iLA active®, Novalung, Heilbronn Germany) had influenza and consecutive acute respiratory distress syndrome (ARDS). One of them had probable pulmonary aspergillosis and was treated with ISA in a standard dosing regimen after sufficient plasma concentrations of voriconazole could not be reached. ISA plasma concentration of this patient was 1.79µg/mL on day 12 of intravenous treatment. He died from a gangrenous bowel on day 14

of the treatment. The other ECMO patient was also treated with a standard dose of ISA in a case of probable intraabdominal *Candida parapsilosis* infection after Caspofungin therapy did not show sufficient response. ISA plasma concentrations were measured on day 1 (during loading dose) and 4 after the beginning of intravenous treatment, and the concentration was 0.74μg/mL and 0.57μg/mL, respectively. After termination of treatment with ECMO, the same patient underwent a second treatment course with ISA. The plasma concentration measured in this treatment course was 2.44μg/mL while still receiving RRT. Eventually, this patient died from secondary sclerosing cholangitis 5 weeks later.

Another patient treated extracorporeally received treatment with Cytosorb® cytokine adsorber built in a continuous RRT circuit for 4 days. He was treated for proven pulmonary aspergillosis with a standard dosing regimen of ISA after Voriconazole had been stopped due to clinical deterioration and toxicity. ISA plasma concentration was measured just before initiation of the treatment and accounted to 1.3μg/mL. In comparison, the concentration measured on the last day of adsorber treatment accounted to 0.82μg/mL. ISA plasma concentrations were also evaluated 14 and 32 days after the termination of cytokine adsorber treatment but ongoing RRT and accounted for 0.62μg/mL and 0.91μg/mL. This patient's response to ISA treatment was defined as being a complete response.

The fourth patient receiving extracorporeal treatment with ECMO was treated for proven pulmonary aspergillosis with a standard dosing regimen of ISA. This patient had developed ARDS after an extensive cardiac intervention. ISA plasma concentration was measured every day for a period of 18 days. The median concentration during ECMO treatment alone was 1.7μg/mL. The patient received additional treatment with RRT on day 12, and ISA plasma concentration was measured to be 0.8μg/mL. After discontinuation of ECMO on day 15, this patient's ISA plasma concentration remained below 0.9μg/mL.

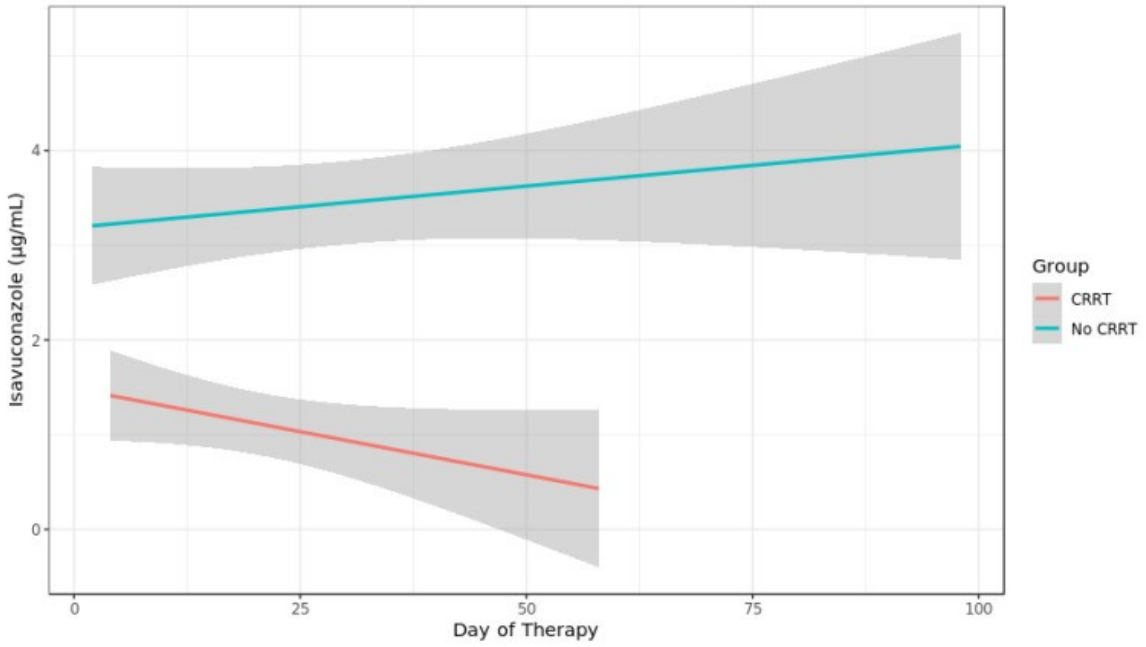

Figure 6. ISA plasma concentration in patients with and without continuous renal replacement therapy (CRRT) (94)

Looking at the six patients (out of 33 ISA treatment courses) encountering adverse events undergoing treatment with ISA, four of them had ISA plasma concentrations measured. Plasma concentrations did not exceed 5.5µg/mL in those patients.ISA plasma concentrations did neither corelate to complete response (see Figure 3) nor to body mass index (see Figure 7).

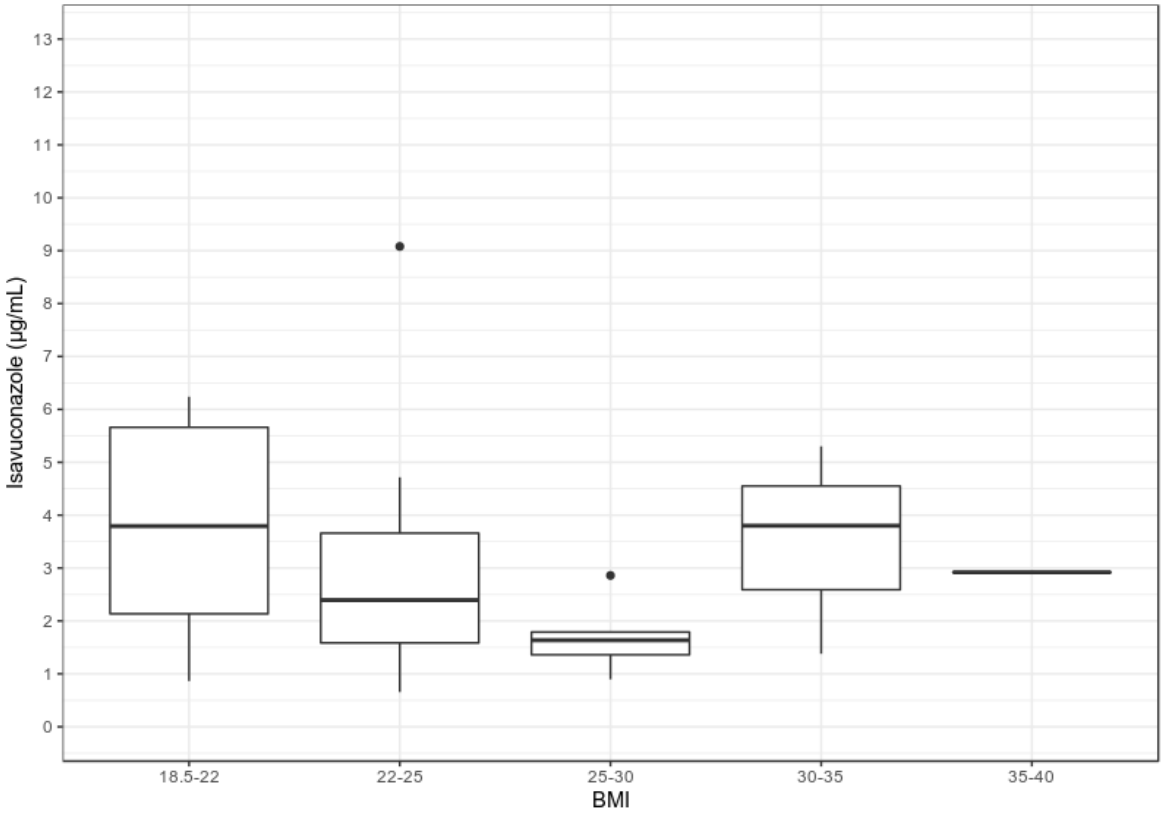

Figure 7. A Correlation between Body-Mass-Index and Isavuconazole (ISA) Trough levels could not be shown.

4 Discussion

Our study cohort consists of patients who suffered from invasive fungal disease with mixed underlying conditions and were treated with ISA. In line with previous studies, we observed that ISA was well-tolerated (1,2,95). Out of 33 treatment courses, adverse events emerged in eighteen percent of the cases, including one with a suspected allergic reaction. Compared to the studies preceding ISAs approval, the rate of adverse events was much lower in our study, e.g., nearly all of the patients (96%) in the SECURE trial experienced at least one TEAE. One study investigating blood levels during prolonged therapy in a routine clinical setting reported TEAE in approximately 30% of their patients (95).

We identified the treatment response as complete and partial response in 87% of our patients. In contrast, nine percent of the patients suffered from progressing fungal infection, eventually leading to death. Like previous studies, we could not identify a threshold in trough concentrations associated with a favorable outcome, most likely due to the low number of treatment failures in our study group and the commonly short duration of their treatment.

Extracorporeal membrane oxygenation (ECMO) is an important treatment option in patients developing ARDS, e.g., following influenza infection. Influenza has been discussed as an independent risk factor for invasive pulmonary aspergillosis (24), high-lightening the importance of choosing the right antifungal treatment in the presence of ECMO therapy. For example, Voriconazole is known to be sequestrated by the ECMO membrane resulting in non-therapeutic plasma levels of the drug (96,97). Micafungin is also discussed to be extracted by ECMO, affecting drug levels in vivo (98). So far, the potential for interaction leading to altered pharmacokinetics of ECMO and ISA has only been illustrated in one case study (99). Two patients from our study group with influenza-associated ARDS required ECMO. They underwent treatment with ISA at the same time, one for IA and the other for probable invasive candidiasis.

Altogether, three patients were treated simultaneously with ECMO and continuous RRT. One patient exhibited an ISA trough level of 1.70µg/mL measured on day 12 of ISA treatment, whereas the second patient showed levels <1µg/mL throughout the treatment with ECMO (trough levels measured on day 1 and 4 of treatment with ECMO; increased to 2.44µg/mL after the termination of ECMO on day 6 but ongoing RRT). A third patient

demonstrated a median ISA plasma concentration of 1.7µg/mL during ECMO, but when RRT was installed additionally, those plasma concentrations decreased.

Clearly, limited by low numbers, these findings support the implication of TDM in patients treated with ISA having concomitant ECMO. This was previously stated for voriconazole and caspofungin. As mentioned above, voriconazole is assumed to be sequestrated within the ECMO circuit, resulting in lower plasma concentrations and based on the plasma levels two of our study patients exhibited during treatment with ECMO, one might assume that ISA plasma concentrations in the early days of parallel treatment are low for the same reason. Additional RRT in these particular patients did not seem to have a scaling effect in plasma levels of ISA.

Critically ill patients being treated with RRT had a median ISA concentration of 1.12µg/mL, which was significantly lower than patients without RRT. In a different study group of critically ill hematology patients with renal impairment suffering from probable invasive aspergillosis it was shown that ISA levels after Sustained Low-Efficiency Dialysis (SLED) were significantly lower due to RRT (100). ISA was not detectable in the ultrafiltrate in the aforementioned study, and therefore, the authors conclude that the adsorption of ISA to the extracorporeal circuit could be assumed (100).

One of the patients included in our study received treatment with Cytosorb® cytokine adsorber four times for 16h each, integrated within one RRT circuit. Cytosorb® contains special polymer beads with a large adsorption surface that eliminates cytokines and metabolic products with a size up to 55 kDa but does not adsorb endotoxins, as they are typically larger than that (101). ISAs has a molecular size of 437.5 Dalton (3). Evaluation of ISA plasma concentrations in the presence of therapy with Cytosorb® suggests that the device might be lowering ISA plasma concentration. This effect's extent and origin remains unclear as our patient demonstrated low plasma concentrations, even 14 and 32 days (0.68µg/mL and 0.91µg/mL) after therapy with cytokine adsorption.

A high BMI was found to impact the clearance of ISA (68).On patient in our study had a BMI of 39.6 kg/m2 (140kg bodyweight), which exceeds previously published BMI and weight standard deviations of published ISA data (1,68). Our patient received a standard dose of ISA to treat probable IA, and his ISA plasma concentrations were measured multiple

times. ISA plasma concentrations were 1, 2.42, 3.68, and 3.42μg/mL, respectively. The treatment outcome was defined as a complete response in this patient.

The exposure-response relationship of ISA as a measure of efficacy and safety in patients with IA and infections by other filamentous fungi has been studied in a post-hoc analysis from the SECURE clinical trial, which showed no statistically significant relationship between ISA exposure and neither efficacy nor safety endpoints. Therefore, it was concluded that ISA exposures achieved by clinical dosing were appropriate for treating the infecting organisms in the SECURE study. Interestingly, side effects were not related to an increase in exposures (84). Plasma concentrations in clinical use and study populations receiving ISA were shown to be comparable (83). Missing patient-level data in this study made it impossible to assess outcome and safety data in relation with ISA plasma concentrations (83). A study evaluating ISA blood levels in 19 hematology patients during prolonged therapy could not establish a cut off level for efficacy but proposed an upper limit of 5μg/mL, because of a relationship between increasing ISA levels and mainly gastrointestinal adverse effects (95).

Calculations regarding treatment failure in in the group of deceased patients were not possible due to the short ISA treatment duration of only 4-10 days. Plasma concentrations of patients who encountered side effects were always below 5.5μg/mL, which is in line with work by others who applied the same dosage and reported no direct proportionality between safety parameters and plasma concentrations (102).

4.1 Limitations and Future Directions

There are some limitations in our study that need to be addressed. First of all, the observational design of the study limits the possibility to rule out systemic biases, such as the problem of confounding or a selection bias. Secondly, the number of patients enrolled in the study and especially, the number of patients analyzed in subgroups is limited, as it is often the case with rare diseases. Clearly, the low number of patients also reduces the statistical significance of our results.

Regarding the real-life designation of our patient cohort it is to argue, if it is truly more diverse than groups of patients enrolled in randomized controlled trials (103).

Given the overall positive results from the use of ISA in its current indications, studies can be expected for the use of this triazole also in anti-mold prophylaxis. Another interesting

field of study might be the broad range of ISAs activity against other molds than *Aspergillus spp*. and Mucorales.

4.2 Conclusion

This thesis outlines a study undertaken in order to evaluate ISA in real-world use. ISA was used in a mixed patient cohort for treatment of invasive fungal diseases and proved not only efficacy, but also good tolerability. Measured plasma concentrations were consistent with those described previously in other trials, even in cases with high body mass index. Patients receiving RRT had significantly lower plasma concentrations than patients without. Also, in patients receiving treatment with ECMO or cytokine absorber plasma concentrations were observed to be low and inconsistent. Therefore, we propose to monitor ISA plasma concentrations in these patients to attain the - to date only putative - therapeutic threshold of >1µg/mL.

4.3 Acknowledgements

Final results of the study were published in *Journal of Fungi*:

Zurl C, Waller M, Schwameis F, Muhr T, Bauer N, Zollner-Schwetz I, Valentin T, Meinitzer A, Ullrich E, Wunsch S, Hoenigl M, Grinschgl Y, Prattes J, Oulhaj A, Krause R. **Isavuconazole Treatment in a Mixed Patient Cohort with Invasive Fungal Infections: Outcome, Tolerability and Clinical Implications of Isavuconazole Plasma Concentrations.** J Fungi (Basel). 2020 Jun 22;6(2):90. doi: 10.3390/jof6020090.

5 Literature

1. 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.
2. Marty FM, Ostrosky-Zeichner L, Cornely OA, Mullane KM, Perfect JR, Thompson GR, et al. Isavuconazole treatment for mucormycosis: A single-arm open-label trial and case-control analysis. Lancet Infect Dis. 2016;16(7):828–37.
3. European Public Assessment Report (EPAR) Cresemba [Internet]. [cited 2020 Aug 18]. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/cresemba
4. FDA approval [Internet]. [cited 2020 Jul 22]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/207500Orig1s000lbl.pdf
5. Slavin MA, Thursky KA. Isavuconazole: A role for the newest broad-spectrum triazole. Lancet [Internet]. 2016;387(10020):726–8. Available from: http://dx.doi.org/10.1016/S0140-6736(15)01218-0
6. Whittaker RH. New concepts of kingdoms of organisms. Science (80-). 1969;
7. Janbon G, Quintin J, Lanternier F, d'Enfert C. Studying fungal pathogens of humans and fungal infections: fungal diversity and diversity of approaches. Genes and Immunity. 2019.
8. Perfect JR. The antifungal pipeline: a reality check. Nat Rev Drug Discov. 2017 Sep;16(9):603–16.
9. Bongomin F, Gago S, Oladele RO, Denning DW. Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision. J fungi (Basel, Switzerland). 2017 Oct;3(4).
10. McNeil MM, Nash SL, Hajjeh RA, Phelan MA, Conn LA, Plikaytis BD, et al. Trends in Mortality Due to Invasive Mycotic Diseases in the United States, 1980–1997. Clin Infect Dis [Internet]. 2001 Sep 1;33(5):641–7. Available from: https://doi.org/10.1086/322606
11. Lass-Flörl C. Epidemiologie von Pilzerkrankungen in Mitteleuropa. Ther Umsch. 2016;73(8):443–7.
12. Hof H, Dörries R. Medizinische Mikrobiologie [Internet]. 6., unverä. Duale Reihe. Stuttgart: Georg Thieme Verlag KG; 2017. p. 498–502. Available from:

https://doi.org/10.1055/b-004-140256

13. Meinhof W, Schirren C. Hans Rieth, 1914–1994. Der Hautarzt [Internet]. 2014;65(11):998–1004. Available from: https://doi.org/10.1007/s00105-014-3502-z
14. Jenks JD, Salzer HJF, Prattes J, Krause R, Buchheidt D, Hoenigl M. Spotlight on isavuconazole in the treatment of invasive aspergillosis and mucormycosis: Design, development, and place in therapy. Drug Design, Development and Therapy. 2018.
15. Guenter S, Gorkiewicz G, Halwachs B, Kashofer K, Thueringer A, Wurm P, et al. Impact of ITS-Based Sequencing on Antifungal Treatment of Patients with Suspected Invasive Fungal Infections. J fungi (Basel, Switzerland). 2020 Mar;6(2).
16. Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, Crokaert F, et al. Defining Opportunistic Invasive Fungal Infections in Immunocompromised Patients with Cancer and Hematopoietic Stem Cell Transplants: An International Consensus. Clin Infect Dis [Internet]. 2002 Jan 1;34(1):7–14. Available from: https://doi.org/10.1086/323335
17. 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 an Off Publ Infect Dis Soc Am. 2020 Sep;71(6):1367–76.
18. Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med. 2012 Dec;4(165):165rv13.
19. Lass-Flörl C. The changing face of epidemiology of invasive fungal disease in Europe. Mycoses. 2009;52(3):197–205.
20. Ullmann AJ, Aguado JM, Arikan-Akdagli 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;
21. Segal BH. Aspergillosis. New England Journal of Medicine. 2009.
22. Ledoux M-P, Guffroy B, Nivoix Y, Simand C, Herbrecht R. Invasive Pulmonary Aspergillosis. Semin Respir Crit Care Med. 2020 Feb;41(1):80–98.
23. Meersseman W, Vandecasteele SJ, Wilmer A, Verbeken E, Peetermans WE, Van Wijngaerden E. Invasive aspergillosis in critically ill patients without malignancy. Am J Respir Crit Care Med. 2004 Sep;170(6):621–5.
24. Schauwvlieghe AFAD, 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 Oct;6(10):782–92.

25. Segal BH, Walsh TJ. Current approaches to diagnosis and treatment of invasive aspergillosis. Am J Respir Crit Care Med. 2006 Apr;173(7):707–17.
26. von Eiff M, Roos N, Schulten R, Hesse M, Zühlsdorf M, van de Loo J. Pulmonary aspergillosis: early diagnosis improves survival. Respiration. 1995;62(6):341–7.
27. Pauw B De, Thomas J. Walsha, Donnellya JP, Stevens D a., Edwards JE, Calandra T, et al. Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive. Clin Infect Dis. 2008;
28. Blot SI, Taccone FS, Van den Abeele A-M, Bulpa P, Meersseman W, Brusselaers N, et al. A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. Am J Respir Crit Care Med. 2012 Jul;186(1):56–64.
29. Patterson TF, Thompson GR, 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):e1–60.
30. Tissot F, Agrawal S, Pagano L, Petrikkos 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.
31. Chong GM, Maertens JA, Lagrou K, Driessen GJ, Cornelissen JJ, Rijnders BJA. Diagnostic Performance of Galactomannan Antigen Testing in Cerebrospinal Fluid. J Clin Microbiol. 2016 Feb;54(2):428–31.
32. Lamoth F, Cruciani M, Mengoli C, Castagnola E, Lortholary O, Richardson M, et al. β-Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). Clin Infect Dis an Off Publ Infect Dis Soc Am. 2012 Mar;54(5):633–43.
33. Herbrecht R, Patterson TF, Slavin MA, Marchetti O, Maertens J, Johnson EM, et al. Application of the 2008 definitions for invasive fungal diseases to the trial comparing voriconazole versus amphotericin B for therapy of invasive aspergillosis: a collaborative study of the Mycoses Study Group (MSG 05) and the European Organization for . Clin Infect Dis an Off Publ Infect Dis Soc Am. 2015 Mar;60(5):713–20.
34. Marr KA, Schlamm HT, Herbrecht R, Rottinghaus ST, Bow EJ, Cornely OA, et al.

Combination antifungal therapy for invasive aspergillosis: a randomized trial. Ann Intern Med. 2015 Jan;162(2):81–9.

35. Cornely OA, Maertens J, Winston DJ, Perfect J, Ullmann AJ, Walsh TJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. N Engl J Med. 2007 Jan;356(4):348–59.
36. Ullmann AJ, Lipton JH, Vesole DH, Chandrasekar P, Langston A, Tarantolo SR, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. N Engl J Med. 2007 Jan;356(4):335–47.
37. Petrikkos G, Skiada A, Lortholary O, Roilides E, Walsh TJ, Kontoyiannis DP. Epidemiology and clinical manifestations of mucormycosis. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2012 Feb;54 Suppl 1:S23-34.
38. Saegeman V, Maertens J, Meersseman W, Spriet I, Verbeken E, Lagrou K. Increasing incidence of mucormycosis in University Hospital, Belgium. Emerg Infect Dis. 2010 Sep;16(9):1456–8.
39. Bitar D, Lortholary O, Le Strat Y, Nicolau J, Coignard B, Tattevin P, et al. Population-based analysis of invasive fungal infections, France, 2001-2010. Emerg Infect Dis. 2014 Jul;20(7):1149–55.
40. No Title [Internet]. [cited 2020 Oct 2]. Available from: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=73263
41. Chakrabarti A, Singh R. Mucormycosis in India: unique features. Mycoses. 2014 Dec;57 Suppl 3:85–90.
42. Reid G, Lynch JP, Fishbein MC, Clark NM. Mucormycosis. Semin Respir Crit Care Med. 2020;41(01):99–114.
43. Jeong W, Keighley C, Wolfe R, Lee WL, Slavin MA, Kong DCM, et al. The epidemiology and clinical manifestations of mucormycosis: a systematic review and meta-analysis of case reports. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2019 Jan;25(1):26–34.
44. Legouge C, Caillot D, Chrétien ML, Lafon I, Ferrant E, Audia S, et al. The reversed halo sign: pathognomonic pattern of pulmonary mucormycosis in leukemic patients with neutropenia? Clin Infect Dis an Off Publ Infect Dis Soc Am. 2014 Mar;58(5):672–8.
45. Chamilos G, Marom EM, Lewis RE, Lionakis MS, Kontoyiannis DP. Predictors of pulmonary zygomycosis versus invasive pulmonary aspergillosis in patients with cancer. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2005 Jul;41(1):60–6.

46. Cornely OA, Alastruey-Izquierdo A, Arenz D, Chen SCA, Dannaoui E, Hochhegger B, et al. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. Lancet Infect Dis. 2019;19(12):e405–21.
47. Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2008 Aug;47(4):503–9.
48. Lanternier F, Poiree S, Elie C, Garcia-Hermoso D, Bakouboula P, Sitbon K, et al. Prospective pilot study of high-dose (10 mg/kg/day) liposomal amphotericin B (L-AMB) for the initial treatment of mucormycosis. J Antimicrob Chemother. 2015 Nov;70(11):3116–23.
49. Lenczuk D, Zinke-Cerwenka W, Greinix H, Wölfler A, Prattes J, Zollner-Schwetz I, et al. Antifungal Prophylaxis with Posaconazole Delayed-Release Tablet and Oral Suspension in a Real-Life Setting: Plasma Levels, Efficacy, and Tolerability. Antimicrob Agents Chemother [Internet]. 2018 Jun;62(6). Available from: https://europepmc.org/articles/PMC5971564
50. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2005 Sep;41(5):634–53.
51. Aktories K. Allgemeine und spezielle Pharmakologie und Toxikologie. 11., übera. München: Elsevier, Urban & Fischer; 2013. p. 814–27.
52. Nivoix Y, Ledoux M-P, Herbrecht R. Antifungal Therapy: New and Evolving Therapies. Semin Respir Crit Care Med. 2020 Feb;41(1):158–74.
53. González GM. In vitro activities of isavuconazole against opportunistic filamentous and dimorphic fungi. Med Mycol. 2009;47(1):71–6.
54. Lepak AJ, Marchillo K, VanHecker J, Diekema D, Andes DR. Isavuconazole pharmacodynamic target determination for Candida species in an in Vivo murine disseminated candidiasis model. Antimicrob Agents Chemother. 2013;
55. Espinel-Ingroff A, Chowdhary A, Gonzalez GM, Guinea J, Hagen F, Meis JF, et al. Multicenter study of isavuconazole mic distributions and epidemiological cutoff values for the cryptococcus neoformans-cryptococcus gattii species complex using the clsi m27-a3 broth microdilution method. Antimicrob Agents Chemother. 2015;

56. Guinea J, Recio S, Escribano P, Peláez T, Gama B, Bouza E. In vitro antifungal activities of isavuconazole and comparators against rare yeast pathogens. Antimicrob Agents Chemother. 2010 Sep;54(9):4012–5.
57. Thompson GR 3rd, Rendon A, Ribeiro Dos Santos R, Queiroz-Telles F, Ostrosky-Zeichner L, Azie N, et al. Isavuconazole Treatment of Cryptococcosis and Dimorphic Mycoses. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2016 Aug;63(3):356–62.
58. Basilea reports acceptance for regulatory review of Pfizer's marketing authorization application for antifungal isavuconazole (Cresemba®) in China [Internet]. [cited 2020 Aug 18]. Available from: https://www.basilea.com/?tx_news_pi1%5Baction%5D=detail&tx_news_pi1%5Bcontroller%5D=News&tx_news_pi1%5Bnews%5D=1217&type=1546938654&cHash=2ac97d7f137981b77436487b0092ffa1#
59. SUMMARY OF PRODUCT CHARACTERISTICS [Internet]. [cited 2020 Jul 22]. Available from: https://www.ema.europa.eu/en/documents/product-information/cresemba-epar-product-information_en.pdf
60. 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 Jun;8(6):759–65.
61. Schmitt-Hoffmann A, Roos B, Heep M, Schleimer M, Weidekamm E, Brown T, et al. Single-ascending-dose pharmacokinetics and safety of the novel broad-spectrum antifungal triazole BAL4815 after intravenous infusions (50, 100, and 200 milligrams) and oral administrations (100, 200, and 400 milligrams) of its prodrug, BAL8557, in health. Antimicrob Agents Chemother. 2006 Jan;50(1):279–85.
62. Townsend R, Kato K, Hale C, Kowalski D, Lademacher C, Yamazaki T, et al. Two Phase 1, Open-Label, Mass Balance Studies to Determine the Pharmacokinetics of 14 C-Labeled Isavuconazonium Sulfate in Healthy Male Volunteers. Clin Pharmacol Drug Dev. 2018;
63. Schmitt-Hoffmann A, Roos B, Maares J, Heep M, Spickerman J, Weidekamm E, et al. Multiple-dose pharmacokinetics and safety of the new antifungal triazole BAL4815 after intravenous infusion and oral administration of its prodrug, BAL8557, in healthy volunteers. Antimicrob Agents Chemother. 2006;
64. Schmitt-Hoffmann A, Desai A, Kowalski D, Pearlman H, Yamazaki T, Townsend R. Isavuconazole absorption following oral administration in healthy subjects is comparable to intravenous dosing, and is not affected by food, or drugs that alter

stomach pH. Int J Clin Pharmacol Ther. 2016;

65. Desai A, Kovanda L, Kowalski D, Lu Q, Townsend R, Bonate PL. Population Pharmacokinetics of Isavuconazole from Phase 1 and Phase 3 (SECURE) Trials in Adults and Target Attainment in Patients with Invasive Infections Due to Aspergillus and Other Filamentous Fungi. Antimicrob Agents Chemother. 2016 Sep;60(9):5483–91.
66. Schmitt-Hoffmann AH, Kato K, Townsend R, Potchoiba MJ, Hope WW, Andes D, et al. Tissue distribution and elimination of isavuconazole following single and repeat oral-dose administration of isavuconazonium sulfate to rats. Antimicrob Agents Chemother. 2017;
67. Schwartz S, Cornely OA, Hamed K, Marty FM, Maertens J, Rahav G, et al. Isavuconazole for the treatment of patients with invasive fungal diseases involving the central nervous system. Med Mycol. 2020 Jun;58(4):417–24.
68. Kovanda LL, Desai A V., Lu Q, Townsend RW, Akhtar S, Bonate P, et al. Isavuconazole population pharmacokinetic analysis using nonparametric estimation in patients with invasive fungal disease (results from the VITAL study). Antimicrob Agents Chemother. 2016;
69. Biagi M, Butler D, Tan X, Qasmieh S, Tejani K, Patel S, et al. Pharmacokinetics and Dialytic Clearance of Isavuconazole during In Vitro and In Vivo Continuous Renal Replacement Therapy. Antimicrob Agents Chemother [Internet]. 2019 Dec 1;63(12):e01085-19. Available from: http://aac.asm.org/content/63/12/e01085-19.abstract
70. Desai A, Schmitt-Hoffmann AH, Mujais S, Townsend R. Population pharmacokinetics of isavuconazole in subjects with mild or moderate hepatic impairment. Antimicrob Agents Chemother. 2016;
71. Viljoen J, Azie N, Schmitt-Hoffmann A-H, Ghannoum M. A phase 2, randomized, double-blind, multicenter trial to evaluate the safety and efficacy of three dosing regimens of isavuconazole compared with fluconazole in patients with uncomplicated esophageal candidiasis. Antimicrob Agents Chemother. 2015 Mar;59(3):1671–9.
72. 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 Jun;101(6):782–90.
73. Astellas Pharma US. Prescribing Information for Isavuconazonium Sulfate. [Internet]. [cited 2020 Sep 3]. Available from:

https://www.astellas.us/docs/cresemba.pdf

74. Horn F. Biochemie des Menschen : das Lehrbuch für das Medizinstudium [Internet]. 3., grundl. Thieme ElectronicBook Library. Stuttgart [u.a.]: Thieme; 2005. p. Online-Ressource (XVIII, 624 S.). Available from: https://dx.doi.org/10.1055/b-002-8316

75. Townsend R, Dietz A, Hale C, Akhtar S, Kowalski D, Lademacher C, et al. Pharmacokinetic Evaluation of CYP3A4-Mediated Drug-Drug Interactions of Isavuconazole With Rifampin, Ketoconazole, Midazolam, and Ethinyl Estradiol/Norethindrone in Healthy Adults. Clin Pharmacol drug Dev. 2017 Jan;6(1):44–53.

76. Nivoix Y, Ubeaud-Sequier G, Engel P, Levêque D, Herbrecht R. Drug-drug interactions of triazole antifungal agents in multimorbid patients and implications for patient care. Curr Drug Metab. 2009 May;10(4):395–409.

77. Yamazaki T, Desai A, Han D, Kato K, Kowalski D, Akhtar S, et al. Pharmacokinetic Interaction Between Isavuconazole and a Fixed-Dose Combination of Lopinavir 400 mg/Ritonavir 100 mg in Healthy Subjects. Clin Pharmacol drug Dev. 2017 Jan;6(1):93–101.

78. Groll AH, Desai A, Han D, Howieson C, Kato K, Akhtar S, et al. Pharmacokinetic Assessment of Drug-Drug Interactions of Isavuconazole With the Immunosuppressants Cyclosporine, Mycophenolic Acid, Prednisolone, Sirolimus, and Tacrolimus in Healthy Adults. Clin Pharmacol drug Dev. 2017 Jan;6(1):76–85.

79. Van Matre ET, Evans SL, Mueller SW, MacLaren R, Fish DN, Kiser TH. Comparative evaluation of isavuconazonium sulfate, voriconazole, and posaconazole for the management of invasive fungal infections in an academic medical center. Ann Clin Microbiol Antimicrob. 2019 Mar;18(1):13.

80. Yamazaki T, Desai A, Goldwater R, Han D, Lasseter KC, Howieson C, et al. Pharmacokinetic Interactions Between Isavuconazole and the Drug Transporter Substrates Atorvastatin, Digoxin, Metformin, and Methotrexate in Healthy Subjects. Clin Pharmacol drug Dev. 2017 Jan;6(1):66–75.

81. Desai A, Yamazaki T, Dietz AJ, Kowalski D, Lademacher C, Pearlman H, et al. Pharmacokinetic and Pharmacodynamic Evaluation of the Drug-Drug Interaction Between Isavuconazole and Warfarin in Healthy Subjects. Clin Pharmacol drug Dev. 2017 Jan;6(1):86–92.

82. Ledoux M-P, Denis J, Nivoix Y, Herbrecht R. Isavuconazole: A new broad-spectrum azole. Part 2: pharmacokinetics and clinical activity. J Mycol Med. 2018

Mar;28(1):15–22.

83. Walsh TJ, Kovanda L, Kitt T, Desai A, Andes D, Zhao M. Isavuconazole Concentration in Real-World Practice: Consistency with Results from Clinical Trials. Antimicrob Agents Chemother. 2018;1–4.
84. Mujais S, Bonate PL, Townsend RW, Kowalski DL, Kovanda LL, Hope WW, et al. Exposure-Response Relationships for Isavuconazole in Patients with Invasive Aspergillosis and Other Filamentous Fungi. Antimicrob Agents Chemother. 2017;61(12):1–9.
85. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs for antifungal agents, version 10.0, 2020. http://www.eucast.org/astoffungi/clinicalbreakpointsforantifungals/. [Internet]. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf. 2020. p. 0–77. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf
86. Segal BH, Herbrecht R, Stevens DA, Ostrosky- Zeichner L, Sobel J, Viscoli C, et al. Defining Responses to Therapy and Study Outcomes in Clinical Trials of Invasive Fungal Diseases: Mycoses Study Group and European Organization for Research and Treatment of Cancer Consensus Criteria. Clin Infect Dis. 2008;47(5):674–83.
87. Wingard JR, Ribaud P, Schlamm HT, Herbrecht R. Changes in causes of death over time after treatment for invasive aspergillosis. Cancer. 2008;
88. Wingard JR. Editorial Commentary: Learning from Our Failures: The Antifungal Treatment Conundrum . Clin Infect Dis. 2008;46(9):1434–5.
89. Shaukat A, Bakri F, Young P, Hahn T, Ball D, Baer MR, et al. Invasive filamentous fungal infections in allogeneic hematopoietic stem cell transplant recipients after recovery from neutropenia: Clinical, radiologic, and pathologic characteristics. Mycopathologia. 2005;159(2):181–8.
90. Ricci P, Nosari A, Tonso A, Buelli M, Montillo M, Cudillo L, et al. Fatal haemoptysis in pulmonary filamentous mycosis: an underevaluated cause of death in patients with acute leukaemia in haematological complete remission. A retrospective study and review of the literature. Br J Haematol. 1995;89(3):500–5.
91. Cornely OA, Hoenigl M, Lass-Flörl C, Chen SCA, Kontoyiannis DP, Morrissey CO, et al. Defining breakthrough invasive fungal infection–Position paper of the mycoses study group education and research consortium and the European Confederation of

Medical Mycology. Mycoses. 2019;62(9):716–29.

92. Jenks JD, Mehta SR, Hoenigl M. Broad spectrum triazoles for invasive mould infections in adults: Which drug and when? Med Mycol. 2019 Apr;57(Supplement_2):S168–78.
93. Pea F, Krause R, Müller C, Hennart B, Richardson M, Meinitzer A, et al. Interlaboratory Analysis of Isavuconazole Plasma Concentration Assays among European Laboratories. Ther Drug Monit. 2019;41(5):657–64.
94. Zurl C, Waller M, Schwameis F, Muhr T, Bauer N, Zollner-Schwetz I, et al. Isavuconazole Treatment in a Mixed Patient Cohort with Invasive Fungal Infections: Outcome, Tolerability and Clinical Implications of Isavuconazole Plasma Concentrations. J fungi (Basel, Switzerland). 2020 Jun;6(2).
95. Furfaro E, Signori A, Di Grazia C, Dominietto A, Raiola AM, Aquino S, et al. Serial monitoring of isavuconazole blood levels during prolonged antifungal therapy. J Antimicrob Chemother. 2019 Aug;74(8):2341–6.
96. Spriet I, Annaert P, Meersseman P, Hermans G, Meersseman W, Verbesselt R, et al. Pharmacokinetics of caspofungin and voriconazole in critically ill patients during extracorporeal membrane oxygenation. J Antimicrob Chemother. 2009 Apr;63(4):767–70.
97. Winiszewski H, Rougny A-C, Lagoutte-Renosi J, Millon L, Capellier G, Navellou J-C, et al. The pharmacokinetic challenge of treating invasive aspergillosis complicating severe influenzae assisted by extracorporeal membrane oxygenation. Vol. 22, Critical care (London, England). 2018. p. 355.
98. Watt KM, Cohen-Wolkowiez M, Williams DC, Bonadonna DK, Cheifetz IM, Thakker D, et al. Antifungal Extraction by the Extracorporeal Membrane Oxygenation Circuit. J Extra Corpor Technol. 2017 Sep;49(3):150–9.
99. Zhao Y, Seelhammer TG, Barreto EF, Wilson JW. Altered Pharmacokinetics and Dosing of Liposomal Amphotericin B and Isavuconazole during Extracorporeal Membrane Oxygenation. Vol. 40, Pharmacotherapy. United States; 2020. p. 89–95.
100. Lahmer T, Batres Baires G, Heilmaier M, Schmid RM, Sörgel F, Kinzig M, et al. Influence of Sustained Low-Efficiency Dialysis Treatment on Isavuconazole Plasma Levels in Critically Ill Patients. Antimicrob Agents Chemother. 2019 Nov;63(11).
101. Schädler D, Pausch C, Heise D, Meier-Hellmann A, Brederlau J, Weiler N, et al. The effect of a novel extracorporeal cytokine hemoadsorption device on IL-6 elimination in septic patients: A randomized controlled trial. PLoS One. 2017;12(10):e0187015.

102. Kaindl T, Andes D, Engelhardt M, Saulay M, Larger P, Groll AH. Variability and exposure-response relationships of isavuconazole plasma concentrations in the Phase 3 SECURE trial of patients with invasive mould diseases. J Antimicrob Chemother. 2019 Mar;74(3):761–7.
103. Gueyffier F, Cucherat M. The limitations of observation studies for decision making regarding drugs efficacy and safety. Therapie. 2019 Apr;74(2):181–5.